Comparative drought resistance of temperate grassland species: mechanisms, prediction and relation to species distribution across moisture gradients

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Table of contents

Abstract

Grasslands are important ecosystems worldwide, exhibiting high biodiversity and providing important ecosystem services. In Europe, they occupy more than one-third of the total agricultural land area. However, grasslands experience periodical drought during the growing season, which reduces their productivity, influences species abundance and distribution, and leads to changes in grassland functions. Climate change is expected to increase the intensity and frequency of drought and thus intensify the impacts of drought on grasslands. To improve our ability to predict the consequences of these changes on grasslands, thorough understandings of species responses to drought, of the traits governing drought responses, and of how they affect community assembly and ecosystem function are imperative. However, lacking comparative datasets on whole-plant drought resistance and comprehensive sets of traits across grassland species currently impedes our understanding of the role of drought under past and current climate regimes, and our projections for the future.

To cover the gaps, in this thesis we comparatively assessed whole-plant drought resistance and an extensive suite of traits. To our knowledge, these are the first comparative datasets across multiple grassland species. With them, we aimed to address the following questions: (1) Do species drought responses vary across species? (2) Do performance trade-offs exist at the whole-plant and trait levels? (3) Are whole-plant drought resistance and traits related to species distribution along moisture gradients? (4) What is the mechanism of drought resistance? (5) Which traits can be used to predict drought resistance?

Whole-plant drought resistance was assessed for 41 common temperate grassland species including 20 forbs and 21 grasses in a common garden drought experiment. The experimental design allowed us to comparatively assess the effects of drought on performances of individual species by only inducing delines in soil water potentials while minimizing effects of other cofounding abiotic and biotic factors that influence plant performances. Additionally, midday leaf water potential under drought was measured to indicate species efficiency of desiccation avoidance. Further, we assessed 38 morphological, anatomical and physiological traits. All traits are hypothesized to be mechanistically related to drought resistance, including ‘hard’ traits that pertain to plant key processes, i.e., water uptake, water storage, water transport, water loss, membrane vulnerability, and carbon gain.

We found that drought significantly reduced species survival and growth, while its effects varied significantly across species. The ranks of species performances remained consistent across drought periods. Many species showed high drought resistance, and a few may suffer greatly under drought. Forbs and grasses had similar whole-plant drought resistance, while they were divergent along trait axes.

Performance trade-offs were not supported. At whole-plant level, growth under irrigated conditions (optimal growth) was unrelated to whole-plant drought resistance of survival and was positively correlated with growth under drought conditions. At trait level, traits that are known to enable high optimal growth had relatively low importance for whole-plant drought resistance. A 'fast-slow' plant economics spectrum did not emerge, suggesting that traits and processes involved in drought resistance and growth under optimal conditions should be independent. Further, whole-plant drought resistance was not related to species distribution along moisture gradients, while drought sensitive species were restricted to wet habitats, suggesting that the relation between drought resistance and species distribution was weak. Consistently, several traits were significantly correlated with species distribution, but they had relatively low importance for whole-plant drought resistance. Together, these results suggest that drought acts as a filter excluding drought sensitive species from dry habitats, but other factors additionally determine species segregation along realized hydrological niches.

The dominance of mechanisms of desiccation avoidance over tolerance was supported. Midday leaf water potential under drought was positively correlated with whole-plant drought resistance. Traits of desiccation avoidance, such as rooting depth, root mass, and stomatal index, had the highest importance for whole-plant drought resistance and species distribution. However, the coordination among traits was complex and no main axes emerged to define a drought resistance spectrum. Many combinations of traits existed to confer grassland species the same or different drought resistance.

Traits can predict species drought resistance. However, a single trait, such as turgor loss point, had relatively low prediction accuracy. Using more root traits and treating forbs and grasses separately, the predictive models were improved ($r^2 \geq 56\%$).

In summary, the differential species drought resistance suggests that increasing drought may have a great impact on temperate grasslands. Our models had relatively high predictive power, which will contribute to predicting the consequences of drought on grasslands and may provide

applicable models for grassland conservation and management under climate change. The comparative datasets of whole-plant drought resistance and traits can allow to further explore of the direct and indirect effects of moisture on community assembly and grassland dynamics at community levels within the framework of the Biodiversity Exploratories.

Zusammenfassung

Grasland gehört aufgrund seiner hohen Biodiversität und der Bereitstellung wichtiger Ökosystemdienstleistungen zu den weltweit wichtigsten Ökosystemen. In Europa handelt es sich bei mehr als einem Drittel der landwirtschaftlichen Fläche um Grasland. In der Vegetationszeit ist Grasland immer wieder Trockenheitsperioden ausgesetzt, die die Produktivität reduzieren, die Abundanz und Verbreitung von Arten beeinflussen und zu Änderungen der Funktion führen. Erwartungsgemäß wird die Intensität und Frequenz von Trockenheitsperioden durch den Klimawandel zunehmen, wodurch sich auch der Einfluss von Trockenheit auf Grasländer verstärkt. Für Vorhersagen über die Konsequenzen dieser Veränderungen auf Graslandgesellschaften sind genaue Kenntnisse nötig, wie Arten auf Trockenheit reagieren, welche Pflanzeneigenschaften diese Reaktion hervorrufen und wie dies die Artenzusammensetzung und die Funktion des Ökosystems beeinflusst. Bisher existieren keine vergleichbaren Datensätze, wie Graslandarten auf Trockenheit reagieren bzw. welche Eigenschaften dafür verantwortlich sind. Dadurch ist unser Verständnis über den Einfluss von Trockenheit auf Grasländer in der Vergangenheit, unter jetzigen klimatischen Bedingungen sowie unsere Prognosen unvollständig.

Um diese Wissenslücken zu schließen, habe ich in dieser Doktorarbeit vergleichende Untersuchungen zu der Trockenresistenz von Graslandpflanzen durchgeführt und eine Vielzahl von Pflanzeneigenschaften gemessen. Nach meinem Kenntnisstand, ist dies der erste umfassende Datensatz für Graslandarten, der sich für Vergleichszwecke eignet. Mit diesem Datensatz habe ich folgende Fragestellungen untersucht: (1) Unterscheiden sich die Arten in ihrer Reaktion auf Trockenheit?; (2) Gibt es Kompromisse („Trade-offs“) zwischen den Eigenschaften und in der Gesamtleistung der Pflanze?; (3) Steht die Trockenresistenz von Arten und deren Eigenschaften in Bezug zu ihrer Verbreitung entlang eines Feuchtgradienten?; (4) Was ist der Mechanismus für Trockenresistenz?; (5) Welche Eigenschaften können zur Vorhersage von Trockenresistenzen genutzt werden?

Es wurde die Trockenresistenz von 41 häufigen temperaten Graslandarten (21 Kräuter, 20 Gräser) in einem kontrollierten Freilandexperiment („Common Garden Experiment“) untersucht. Ich habe das Wasserpotenzial der Blätter zur Mittagszeit („Midday Leaf Water“ Potenzial) unter Trockenstress gemessen, welches auf eine effiziente Austrocknungsvermeidung hinweist. Darüber hinaus habe ich 38 morphologische,

physiologische und anatomische Pflanzeneigenschaften und deren phänotypische Plastizität untersucht. Alle Eigenschaften (u.a. „Hard Traits“) beeinflussen potenziell die Trockenresistenz, die mit Schlüsselprozessen wie Wasseraufnahme, Wasserspeicherung, Wassertransport, Wasserverlust, Durchlässigkeit der Membran und Kohlenstoffaufnahme, in den Pflanzen in Zusammenhang stehen. Der experimentelle Aufbau erlaubte es die Leistung einzelner Arten allein auf die Abnahme des Wasserpotenzials im Boden zurückzuführen, ohne weitere biotische und abiotische Faktoren berücksichtigen zu müssen.

Die Arten reagierten unterschiedlich auf Trockenheit, welche eindeutig das Überleben und Wachstum der Arten verringerte. Die Reihenfolge der Artenleistung bei verschiedenen Trockenperioden blieb konstant. Viele Arten zeigten eine hohe Trockenresistenz und nur wenige litten stark unter der Trockenheit. Kräuter und Gräser hatten eine ähnliche Trockenresistenz, aber variierten in ihren Eigenschaften.

Trade-offs in der Leistung der Pflanzen traten nicht auf. Zwischen der Wachstumsrate unter bewässerten Bedingungen und dem Wachstum unter Trockenheit bestand eine positive Korrelation, jedoch bestand kein Zusammenhang zur Überlebensrate unter Trockenheit. Pflanzeneigenschaften, die ein schnelles Wachstum ermöglichen, hatten einen geringeren Einfluss auf die Trockenresistenz. Ein „schnell-langsam“ Wirtschaftsspektrum („slow-fast economic spectrum“) trat nicht auf. Das heißt, unter optimalen Wachstumsbedingungen sind die Eigenschaften und Prozesse, welche Wachstum und Trockenresistenz bestimmen, unabhängig. Die Trockenresistenz stand in keiner Beziehung zu der Verbreitung der Arten entlang eines Feuchtegradienten. Trockensensitive Arten waren jedoch auf feuchte Habitate beschränkt. Eigenschaften, die mit der Verbreitung der Arten in Zusammenhang standen, hatten einen geringen Einfluss auf die Trockenresistenz. Zusammenfassend, deuten diese Ergebnisse daraufhin, dass Trockenheit als Filter wirkt, der trockenheitssensitive Arten von trockenen Habitaten ausschließt. Jedoch bestimmen nicht „Trade-offs“ zwischen den Eigenschaften und der Trockenresistenz, sondern andere Faktoren die Verteilung der Arten entlang realisierter hydrologischer Nischen.

Mechanismen für die Vermeidung von Trockenheit überwiegen gegenüber denen für Trockentoleranz. „Midday Leaf Water“ Potenziale unter Trockenheit korrelierten positiv mit der Trockenresistenz. Eigenschaften, die der Vermeidung von Austrocknung dienen, wie Wurzeltiefe, Wurzelmasse und Stomata-Index, hatten den höchsten Einfluss auf die Trockenresistenz und Artverbreitung. Allerdings ist das Zusammenspiel zwischen den

verschiedenen Eigenschaften komplex und es gibt keine Hauptachsen, die ein Trockenresistenzspektrum definieren würden. Stattdessen existieren zahlreiche Kombinationen von Eigenschaften, die dazu führen, dass verschiedene Graslandarten eine ähnliche, geringere oder höhere Trockenresistenz besitzen.

Pflanzeneigenschaften erklärten die Trockenresistenz. Allerdings hatte eine einzelne Eigenschaft, wie der Welkepunkt, nur eine sehr geringe Vorhersagekraft. Durch die Nutzung weiterer Wurzeleigenschaften und der separaten Berücksichtigung von Gräsern und Kräutern konnte die Vorhersagekraft der Modelle verbessert werden ($r^2 \geq 56\%$).

Insgesamt verweist die unterschiedliche Reaktion der Arten darauf, dass zunehmende Trockenheit einen großen Einfluss auf temperate Grasländer haben wird. Obwohl verschiedene Eigenschaften zu einer hohen Trockenresistenz führen, können diese Eigenschaften zur Vorhersage von artspezifischen Trockenresistenzen genutzt werden. Dies ermöglicht es die Konsequenzen von Trockenheit vorherzusagen und dient der Entwicklung anwendungsorientierter Modelle für die Erhaltung und das Management von Grasländern unter Klimawandel. Umfassende Datensätze über die Trockenresistenz von Arten und Eigenschaften erlauben es den direkten und indirekten Einfluss von Feuchtigkeit auf die Zusammensetzung der Artengemeinschaft und deren Dynamik im Rahmen der Biodiversitäts-Exploratorien abzuschätzen.

Synopsis

Backgrounds

Grasslands and drought

Grasslands occupy one-fifth of the world's land surface (Scurlock & Hall, 1998), contain the highest biodiversity at small scales in the world (≤ 50 m; Wilson *et al.*, 2012), and store one-third of terrestrial carbon (Gibson, 2009). In Europe, they cover 8% land surface and 35% agricultural area, and provide important goods and services (Smit *et al.*, 2008). However, grasslands experience periodical drought during the growing season (Gibson, 2009). In major grasslands around world, such as North America's prairie (Tilman & El Haddi, 1992; Harrison *et al.*, 2015; Konings *et al.*, 2017), European grasslands (Buckland *et al.*, 1997; Stampfli & Zeiter, 2004), savanna grasslands (O'Connor, 1995), and Asian grasslands (Kinugasa *et al.*, 2012), natural drought has been reported to reduce species abundance, lead to species loss and changes in grasslands species and functional compositions, and impair ecosystem services (Tilman & El Haddi, 1992; Buckland *et al.*, 1997; Stampfli & Zeiter, 2004; Frank, 2007; Harrison *et al.*, 2015; Konings *et al.*, 2017). Under global climate change scenarios, increasing temperature and drought are projected to occur in grasslands during the growing season (IPCC, 2014; Schlaepfer *et al.*, 2017), strengthening the impacts of drought on grasslands. Therefore, it is urgently needed to improve our ability to predict the consequences of drought on grasslands. However, relevant comparative whole-plant drought resistance datasets are mostly restricted to woody species (Kursar *et al.*, 2009; O'Brien *et al.*, 2014; Anderegg *et al.*, 2016). The few studies focusing on grassland species are confined to a limited number of species (4-8 species; Fernandez & Reynolds, 2000; Perez-Ramos *et al.*, 2013; Zwicke *et al.*, 2015). Lacking comparative whole-plant drought resistance datasets across multiple species hinders our understandings of species responses to drought, of the traits governing drought responses, and of how they affect community assembly and ecosystem function, and thus limits our projections for the future.

In the thesis, we comparatively assessed whole-plant drought resistance across 41 common temperate grassland species, including 20 forbs and 21 grasses. We further measured a

comprehensive of traits, i.e., 38 morphological, anatomical and physiological traits. All traits have mechanistic hypotheses on relations with drought resistance, including 'hard' traits that pertain to key processes in plants, i.e., water uptake, water storage, water transport, water loss, membrane vulnerability, and carbon gain. This is the first big dataset of comparative species whole-plant drought resistance and of an extensive suite of traits for grassland species. We aimed to understand the role of drought and traits in determining species distribution, to increase our understandings of drought resistance and its mechanisms, and to improve our predictions of consequences of drought on grasslands. This study was conducted within the framework of the Biodiversity Exploratories which provides data on vegetation composition and moisture variation and allows to further evaluate the relative importance of drought and traits for community assembly (Fischer *et al.*, 2010).

Drought resistance and species distribution

Scientists have long recognized the differential preferences of species to the physical environment (Grinnell, 1904). Compared with other factors, species tolerances and requirements for certain environmental conditions are hypothesized to be the most important determinants of species distribution patterns (Barve *et al.*, 2011). Two hypotheses regarding to performance trade-offs can be used to explain species sorting along environmental gradients. The 'growth-stress tolerance' trade-off hypothesizes that species with low maximum potential growth rate are stress tolerant and maintain high survival under environmental stress (Grime & Hunt, 1975). Later work has extended it to a trait-based spectrum, i.e., the leaf economics spectrum or the 'fast-slow' plant economics spectrum, hypothesizing that traits indicating low growth rate, such as low specific leaf area, low maximum photosynthesis, low specific root length and high root tissue density, are coordinated with each other and confer high stress tolerance capacity (Wright *et al.*, 2004; Reich, 2014). The 'growth rate' trade-off suggests that species have higher growth rate under high resource levels will exhibit lower growth rate at low resource levels than their counterparts with lower growth rate under high resource levels (Latham, 1992). Both hypotheses have been hypothesized to be crucial and general trade-offs and extensively studied regarding to light and nutrient in woody species (Sack & Grubb, 2001; Wright *et al.*, 2010; Reich, 2014). However, despite their pervasive implications for species distribution, competition and community assembly, their pertinence to drought, in contrast, have been rarely empirically investigated. At whole-plant level optimal growth was found to

be unrelated to whole-plant drought resistance of survival (Fernandez & Reynolds, 2000) and positively, rather than negatively, correlated with growth under drought (Reader et al. 1993). Consistently, at trait level, Craine *et al.* (2013) found that physiological drought resistant species had higher maximum photosynthesis. These results suggest that no trade-offs exist between optimal growth and survival or growth under drought.

Yet, across moisture gradients, species drought resistance has been shown to shape species distribution in tropical forests (Engelbrecht *et al.*, 2007). It was also suggested to determine species segregation along hydrological niches in grassland species (Silvertown *et al.*, 1999). However, a direct evaluation of the association of whole-plant drought resistance with species distribution has not yet been tested in grassland species. Several studies have tested this by relating traits to species distributions, such leaf water potentials at stomatal closure, specific leaf area or gas exchange traits, under the assumptions that they are main drivers of differential drought performance (Tucker *et al.*, 2011; Craine *et al.*, 2013; Belluau & Shipley, 2017; Shipley *et al.*, 2017). Overall, these studies showed weak associations between traits and species distributions along moisture gradients suggesting that a weak soring effect of drought on grassland species and their traits. However, our understanding of trait associations with whole-plant drought resistance remains limited. The weak relations between traits and species distribution along moisture gradients could be caused by the poor relations of these traits with whole-plant drought resistance.

In this thesis, we aimed to understand the role of drought and trait on species distribution. We specifically addressed the questions: (1) What are the performances of species under drought? (2) Do performance trade-offs exist at whole-plant and trait levels? (3) Are whole-plant drought resistance and traits related to species distribution along soil moisture?

Mechanisms of drought resistance

Mechanisms of drought resistance, i.e,. mechanisms that allow plants to withstand periods of low water availability, are usually grouped into two main groups: desiccation avoidance and desiccation tolerance (Levitt, 1972). Desiccation avoidance is characterized by high plant water potentials under drought (Levitt, 1972). The efficiency of desiccation avoidance thus can be assessed by midday leaf water potential under drought, which integrates plant traits at root, stem and leaf levels (Levitt, 1972; Comita & Engelbrecht, 2014; Fang & Xiong, 2015). Desiccation tolerance enables plants to maintain physiological activities and functions even

under severe water stress. Traits confer high efficiency of desiccation avoidance include (1) root traits that maximize water uptake, (2) high water storage in plant organ, and (3) traits that minimize transpirational water loss through leaves such as high turgor loss point, high leaf water potentials at stomatal closure, low stomatal and cuticular conductance, high stomatal density and index, low stomatal size and low specific leaf area (Levitt, 1972; Comita & Engelbrecht, 2014; Fang & Xiong, 2015). Traits belonging to desiccation tolerance (1) allow water transport and gas exchange under low water potentials such as high xylem resistance to embolism, high leaf vein density, and insensitive stomatal response to water stress through low turgor loss point, and (2) enable cell membrane structure to keep integral under low tissue water potentials (Levitt, 1972; Comita & Engelbrecht, 2014; Fang & Xiong, 2015). These traits may all contribute to drought resistance with their importance likely to differ (Choat *et al.*, 2018). However, an evaluation of the relative importance of these traits are missing in both woody and herbaceous species due to the lack of datasets on comparative whole-plant drought resistance and a comprehensive set of traits, impeding our understanding of mechanisms of drought resistance.

The interactions among traits are suggested to be complex and may enable many combinations of traits to resist drought (Choat *et al.*, 2018). For example, two studies on grassland species found that although high rooting depth was important for drought resistance, species with shallow roots can still maintain high leaf water potentials and water contents under drought (Garwood & Sinclair, 1979; Buckland *et al.*, 1997), suggesting other traits contributed to drought resistance in these species. Yet, traits may function in a coordinated way due to physiological linkages and natural selections. For instance, traits pertaining to water loss, such as turgor loss point and leaf water potentials at stomatal closure, were positively related (McAdam & Brodribb, 2015; Bartlett *et al.*, 2016) and both were coordinated with water transport in roots, stems, and leaves in woody species (Bartlett *et al.*, 2016). These coordination among traits may enable trait interactions collapse into main axes, such as a ‘fast-slow’ plant economics spectrum (see above) or a hydraulic spectrum in woody species (Manzoni, 2014; Bartlett *et al.*, 2016), but remains to be tested in grassland species.

To improve our understanding of mechanisms of drought resistance, we assessed the importance of traits and midday leaf water potential under drought for whole-plant drought resistance, evaluated trait relations and tested if main axes among trait variations emerged.

Predictions of species drought resistance using traits

Traits can be used to predict species performance under certain environmental conditions due to their associations with plant functions or as functions themselves (Violle *et al.*, 2007). They have been used to predict species performance under natural conditions (Herz *et al.*, 2017; Medeiros *et al.*, 2018), to explain community assembly and species distributions (Kraft *et al.*, 2008; Diaz *et al.*, 2009), and to predict community responses to climate (Lavorel & Garnier, 2002). The fact that traits are relatively easy to measure and confer the generality and predictivity motivates the use of traits as proxies of comparative drought resistance (O'Brien *et al.*, 2017). Turgor loss point, i.e., the leaf water potentials at which leaf cells lose turgor, has been suggested as a key trait to predict species responses under drought (Blackman, 2018). It was significantly correlated with species survival under drought (Zhu *et al.*, 2017) and other leaf water relation traits that are important for drought resistance, such as xylem resistance to embolism or leaf water potential at stomatal closure (Bartlett *et al.*, 2016). A recent method that assesses turgor loss point with osmotic water potential at full turgor using osmometer accelerates assessment of turgor loss point and is applicable to various leaf types, making the use of turgor loss point even more appealing. For grassland species, however, few studies assessed turgor loss point and found it was not related to species distribution (Ocheltree *et al.*, 2016). No studies have yet tested its association with whole-plant drought resistance in grassland species. An evaluation of the association of turgor loss point with whole-plant drought resistance across multiple grassland species thus is needed to assess the use of turgor loss point for predicting species drought resistance and grassland dynamics under drought.

Root traits have been suggested to be more appropriate for predicting drought resistance than turgor loss point and other leaf level traits because many grassland species showed rapid leaf death under drought and can survive drought after complete loss of aboveground tissue (Volaire *et al.*, 1998). Consistently, root traits, such as rooting depth and root mass, were found to significantly contribute to high growth and survival under drought (Perez-Ramos *et al.*, 2013; Zwicke *et al.*, 2015). These results suggest that including root traits may be necessary to improve our prediction accuracy.

In this thesis, we assessed the predictive power of turgor loss point and root traits for whole-plant drought resistance of survival. The models based on these traits might improve our predictions of consequences of drought on grasslands.

Materials and Methods

Study species

41 temperate grassland species, 20 forbs and 21 grasses, were initially chosen according to the following criteria: (1) common in German grasslands based on their high abundance and frequency in 150 long-term grassland plots (4m × 4m) in Biodiversity Exploratories in a three areas across Germany (Schorfheide-Chorin, Hainich-Dün and Schwäbische Alb; Fischer *et al.*, 2010), (2) to include grasses and forbs, (3) association to a wide range of soil moisture based on Ellenberg's soil moisture index (M-values from 3 to 7, Ellenberg *et al.*, 1991), and (3) being perennial and C3, the dominant life history strategy and photosynthetic pathway in European temperate grasslands (Ellenberg *et al.*, 1991; Collatz *et al.*, 1998). The selected species were from 33 genera and 10 families, i.e., covered a wide range of phylogenetic backgrounds.

Experiments

Two experiments, a common garden drought-irrigation experiment and a greenhouse experiment, were carried out to assess species performances under drought and irrigated conditions, midday leaf water potential under drought, and 38 morphological, anatomical and physiological traits. All traits are associated with key processes in plants, i.e., water uptake, water storage, water transport, water loss, membrane vulnerability and carbon gain, and have been known or hypothesized to be important for drought resistance.

The design of the common garden experiment aimed to expose all species to uniformly pronounced drought to assess drought performances of the individual species that are directly comparable across all species, rather than to mimic climate extreme events or a specific natural drought. Other cofounding abiotic and biotic factors that affect plant performances were minimized by randomly planting one individual per species into each plot in a 20 cm grid to avoid overlaps between roots and between leaves. Plots were fertilized twice before the drought treatment to avoid potential nutrient limitation. This is contrasted with field observational data or data from experimental drought in natural or manipulated plant communities where interactions among plants or the influences of other environmental factor, such as nutrient or light, may blur the drought effects (Beier *et al.*, 2012). It also overcomes the potential problems in pot experiments that the different transpiration rate caused by plant size and species-specific

stomatal responses can drawdown soil water in different pots differentially and hinder the comparison across species (Comita & Engelbrecht, 2014).

Drought performances were measured under experimental drought and irrigated conditions in the common garden. Whole-plant drought resistance was assessed as the response ratio of survival and growth under drought relative to irrigated conditions. Midday leaf water potentials under drought were measured when most plants wilted. To ensure comparativeness across species, all individuals within one plot were measured each day, i.e., one individual per species each day.

We used Ellenberg's indicator values for moisture (M-values) as an index of species distribution across moisture gradients (Ellenberg *et al.*, 1991). M-values has been shown to be highly correlated with actual quantification of habitat water availability in Europe and be a reliable indicators of species association with habitat dryness (Diekmann, 2003; Schaffers & Sýkora, 2009).

Most traits were assessed under well-watered conditions in the greenhouse and the common garden experiments. The assessment of these trait was without reference to the specific environmental background and conferred the comparativeness across studies (Violle *et al.*, 2007). However, many of traits express high phenotypic plasticity such as turgor loss point (Bartlett *et al.*, 2014) and root traits (Comas *et al.*, 2013). Ignorance of traits responses to drought may underestimate the importance of traits and the phenotypic plasticity itself. We, therefore, assessed root allocation traits and their phenotypic plasticity under drought conditions. For other traits, however, the assessments of trait plasticity will either interrupt our drought experiment or destruct our plants greatly. Overall, we found that traits in drought conditions are mainly determined by its potential values at optimal conditions or irrigated conditions (see below). The role of phenotypic plasticity is thus limited.

Manuscripts of this thesis

This thesis is composited by four manuscripts, each presenting different datasets and addressing different questions that we aimed to solve. In Manuscript 1, we assessed species performances under drought and irrigated conditions. We tested the performance trade-offs at whole-plant level and evaluated the relation of whole-plant drought resistance with species distribution along moisture gradients. The dataset in Manuscript 1 provides the basis to assess mechanisms of drought resistance, trait importance and the use of traits for predictions. In Manuscript 2 and Manuscript 3, we assessed the importance of mechanisms of desiccation avoidance for whole-plant drought resistance of survival, and the prediction power of traits. In Manuscript 4, we used a comprehensive set of traits to test if performance trade-offs are supported at trait level, i.e., if a 'fast-slow' plant economics spectrum emerges, to assess the relations of traits with species distribution, and to evaluate trait coordination and the relative importance of traits for drought resistance. The differences between forbs and grasses in traits, drought resistance and predictive models were evaluated from Manuscript 1-4. These studies contributed to improve our understanding of the role of drought and traits on species distribution, to increase our understandings of drought resistance and its mechanisms, and to improve our predictions of consequences of drought on grasslands. The results from each manuscript were summarized below.

Manuscript 1: Comparative drought resistance and its relation with species distribution

Drought significantly reduced plants growth and survival, but its effect varied across all species as well as across forbs and grasses. Species ranks of drought resistance held under a wide range of drought intensities as indicated by the positive correlations between species survival after different drought periods and the final survival. Many forbs and grasses could efficiently resist drought with more than 70% species having survival > 90%, a few showed relatively low drought resistance. This could lead to changes in grassland composition and functions under drought due to the loss of drought sensitive species and invasion of exotic species or the expansion of local drought resistance species. Overall, forbs and grasses showed similar whole-plant drought resistance. This study provided comparative quantifications of whole-plant drought resistance which allows us to test the mechanisms of drought resistance and assess the importance of traits for different drought performance parameters and the use of traits for the predictions.

Growth under irrigated conditions was unrelated to whole-plant resistance of survival, and weakly positively correlated with growth under drought, indicating that the performance trade-offs were not supported at whole plant level. Further, whole-plant drought resistance of both survival and growth were not related to species association with habitat dryness. However, the most drought sensitive species (e.g. whole-plant drought resistance of survival < 0.8) were restricted to wet habitats. These results suggest that drought acts as a filter excluding drought sensitive species from dry habitats, but other abiotic and biotic factors additionally influence species segregation along the realized hydrological niches.

Manuscript 2: The importance of desiccation avoidance and the use of turgor loss point for predicting whole-plant drought resistance of survival

In Manuscript 2, three parameters in terms of survival, i.e., drought survival (dead or alive in dry treatment), % survival (drought survival/initial number in dry treatment), and response ratio (% survival in dry relative to irrigated treatment), were used to assess species drought resistance. Forbs had significantly higher turgor loss point and maintained higher midday leaf water potential under drought and higher drought survival than grasses, but both had similar response ratios. To keep consistent, only results from response ratio, i.e., whole-plant drought resistance of survival, were presented.

We directly related midday leaf water potential under drought and turgor loss point to whole-plant drought resistance of survival. Significantly positive correlations between midday leaf water potential under drought and whole-plant drought resistance of survival across all species as well as across forbs and grasses suggest that mechanisms of desiccation avoidance were important for grassland species to resist drought. This was supported by a significantly positive effect of turgor loss point on whole-plant drought resistance of survival and by that the effect was mediated by midday leaf water potential under drought. These results showed that turgor loss point is a key trait for desiccation avoidance and drought resistance in grassland species. However, direction of the effect of turgor loss point on drought resistance in grassland species was opposite to in woody species that a low turgor loss point contributed to high drought resistance. Nevertheless, the explained variance of whole-plant drought resistance by turgor loss point was low. Including other important mechanisms of desiccation avoidance is necessary for the prediction.

Manuscript 3: Root traits contributed to whole-plant drought resistance of survival and improved our prediction

In this manuscript, we included root traits that are known or hypothesized to be important for water uptake. Rooting depth and root mass in dry treatment plots were significantly correlated with and had higher importance than other root traits and turgor loss point for whole-plant drought resistance, suggesting that maximize water uptake from deep soils under drought were more important for drought resistance. Both traits were also significantly correlated with midday leaf water potential under drought supporting the importance of mechanisms of desiccation avoidance in grassland species, consistent with results from Manuscript 2.

Low root tissue density has been suggested to enable high root length per unit biomass allocated (i.e. high root specific length) and thus increase plants water uptake under drought. Opposite to this expectation, high root tissue density promoted high midday leaf water potential under drought. High root tissue density can reflect high number of small and highly lignified xylem vessels that are resistant to drought-induced embolism. It may thus indicate a role of mechanisms of desiccation tolerance in promoting high midday leaf water potentials under drought in grassland species.

Root traits under drought conditions were significantly correlated with trait values under irrigated conditions, suggesting that species water uptake capacity was largely determined by inherent root characteristics. Phenotypic plasticity of root traits overall showed weak correlations with whole-plant drought resistance of survival and midday leaf water potential under drought, and had relatively low importance.

Many root traits were significantly different between forbs and grasses. The traits had the highest importance for whole-plant drought resistance of survival and midday leaf water potential under drought were also different between both groups.

The inclusion of important root traits increased our predictive power. When forbs and grasses were treated separately, root morphological traits, i.e., root diameter, root tissue density and root specific length, can explain 56% variance in forbs, and root mass and rooting depth in irrigated treatment plots and root depth distribution explained 60% in grasses. These traits were assessed under well-watered conditions which facilitates the applications of these models.

Manuscript 4: Mechanisms of drought resistance and trait relations to species distribution

Mechanisms of desiccation avoidance were dominant for drought resistance and distribution in grassland species. Traits of desiccation avoidance, rooting depth and root mass under drought conditions and stomatal index, had the highest importance for whole-plant drought resistance of both survival and growth and for species associations to habitat dryness. These results support the use of root traits to predict whole-plant drought resistance of survival in Manuscript 3. In contrast, few traits of desiccation tolerance were significantly correlated whole-plant drought resistance and species distribution but had relatively low importance.

Traits were coordinated with each other, while many trade-offs and independence existed. This complicated trait relations enabled many different trait combinations to promote drought resistance. No dominating axes of trait variation emerged including the 'fast-slow' plant economics spectrum. Traits measured in our study that are supposed to align on the 'fast-slow' plant economics spectrum were maximum photosynthesis, leaf nitrogen content, specific leaf area, specific root length, root tissue density, and root diameter. However, most of them varied independently. Nevertheless, these results were consistent with results in Manuscript 1, namely, at whole-plant level the performance trade-offs were not supported.

Different sets of traits contributed to drought resistance and species distribution. Traits that were significantly correlated with species association to habitat dryness were unrelated to whole-plant drought resistance and midday leaf water potential under drought. Consistently, stomatal index which had the highest importance for species association to habitat dryness had relatively low importance for whole-plant drought resistance and midday leaf water potential under drought. These results were in line with the weak relation of whole-plant drought resistance with species distribution from Manuscript 1, suggesting that other factors together with drought determine grassland species distribution across moisture gradients.

Consistent with results from Manuscript 3, we found forbs and grasses significantly differed in most of the traits besides root traits and turgor loss point. These differences were strong enough to separate them along trait axes. Further, different combinations of traits promoted drought resistance in both groups.

General discussion

In the following section, I discussed the results from different manuscripts in view of the role of drought and trait on species distribution, mechanisms of drought resistance, and prediction of drought resistance. I further presented some results that went beyond current thesis and linked whole-plant drought resistance and traits datasets to species distribution and responses to drought at community level.

Drought resistance and species distribution

Performance trade-offs have been used to explain species distribution along light and nutrient gradients (Sack & Grubb, 2001; Wright *et al.*, 2010; Reich, 2014). We expected that they would shape species sorting across moisture gradients, i.e., on one hand, species with inherent high growth rate can eliminate drought resistant species from wet habitats due to competition; on the other hand, they will be excluded from dry habitats due to low drought resistance or growth rate under drought. However, at both whole-plant and traits levels, we found no indication for performance trade-offs. Optimal growth was not negatively correlated with whole-plant drought resistance of survival or growth under drought (Manuscript 1). Traits that are hypothesized to enable 'fast' growth showed no or weak correlations with whole-plant drought resistance of both survival or growth (Manuscript 4). The 'fast-slow' plant economics spectrum did not emerge (Manuscript 4). Consistently, at whole-plant level, Fernandez and Reynolds (2000) also found no trade-off between maximum potential growth and whole-plant drought resistance of survival in desert grassland species. Reader *et al*. (1993) found a positive correlation between growth under irrigated and drought conditions in grassland species. At trait level, although the 'fast-slow' plant economics spectrum was found at global scale (Wright *et al.*, 2004), at regional and local scales no supports were found (Craine *et al.*, 2001; Tjoelker *et al.*, 2005; Tucker *et al.*, 2011; Maire *et al.*, 2012; Perez-Ramos *et al.*, 2013; Bergmann *et al.*, 2017; Zhou *et al.*, 2018). In contrast, Grime *et al.* (2000) found that a limestone grassland composed of 'fast-growing' species were more sensitive to drought in term of species richness and functional composition than of 'slow-growing' species. However, the classification of species into fast- or slow-growing groups in Grime *et al.* (2000) was based on S values rather than actual growth rate, i.e., stress tolerance value of the CSR triangle (competitor, stress-tolerator, and ruderal) using leaf morphological traits (Hodgson *et al.*, 1999). The associations of S values with optimal growth and drought resistance are overall weak. In our grassland

species, S values were unrelated to optimal growth or whole-plant drought resistance (Jung unpublished data). In woody species, S values were not related to traits indicating 'fast' growth or high drought resistance such as leaf longevity, leaf nitrogen content and turgor loss point (Rosado & de Mattos, 2017). Collectively, these results suggest that a trade-off between 'fast' growth and drought resistance is not supported, and traits and processes involved in drought resistance and growth under optimal conditions should be independent.

Consistent with the lack of a trade-off between optimal growth and drought resistance, the role of species drought resistance in shaping species distribution across moisture gradients was limited. Drought sensitive species was excluded from dry habitats due to drought while drought resistant species were associated with a wide range of moisture gradient (Manuscript 1). These results suggest that other factors additionally determine species distribution across moisture gradients, but with no trade-offs with drought resistance. This is in line with our results with traits, namely stomatal index had the highest importance for species distribution and highly correlated with it ($r = 0.64$, $P < 0.001$), while it had relatively low importance for whole-plant drought resistance (Manuscript 4). Consistently, previous study also found the positive relation between stomatal index and species association to habitat dryness (Salisbury, 1927). Stomatal index was found to response to both water availability (Xu & Zhou, 2008) and light (Lake *et al.*, 2001). The later is another important factor that influences grassland species distribution (Borer *et al.*, 2014). Thus, along the moisture gradients, changed light regimes might additionally determine species segeragaion along realized hydrological niches, but the association of stomatal index with species distribution across light gradients, and of drought resistance with shade resistance remain to be assessed.

This thesis provided the first comparative datasets on whole-plant drought resistance of both survival and growth and a comprehensive of traits that are hypothesized or known to be important for drought resistance. We attempted to combine these data with Biodiversity Exploratory data on vegetation composition and moisture variation in the experimental plots (Fischer *et al.*, 2010) to evaluate the relative importance of drought for species distribution and community assembly. Although such an approach is powerful to assess the role of resource availability in shaping communities, it has so far rarely been applied. Our preliminary results showed that community weighted means of whole-plant drought resistance and trait values that conferred high drought resistance increased with decreasing soil moisture in relatively dry sites, but not in wet sites. These results support that drought acts as a filter to exclude drought

sensitive species from dry habitats but only in dry areas. In wet areas, other factors are more importance.

Mechanisms of drought resistance

Evaluating trait importance for species performance under certain environmental conditions is one of the main schemes of functional ecology (Shipley *et al.,* 2016). Regarding to drought, multiple processes are involved in drought resistance, which in turn multiple traits with their roles likely to differ with increasing drought stress (Choat *et al.,* 2018). However, the relative importance of these traits for drought resistance has rarely been assessed due to lack of a comprehensive traits dataset. Here we found that root traits that maximize water uptake from deep soil layers, such as rooting depth and root mass, were more important than others (Manuscript 4). These results support the argument in Volaire *et al.* (1998) that root traits were important than leaf traits for grassland species because leaf death under drought in grassland species was rapid and any role of leaf traits such as turgor loss point to minimize water loss was limited. However, loss of leaves itself is an important mechanism to reduce water loss under drought. Many individuals of our species resprouted with complete leaf mortality in the following spring after the drought treatment (Manuscript 1). Further, many leaf traits did contribute to whole-plant drought resistance (Manuscript 4). Nevertheless, by assessing the importance of various traits of desiccation avoidance and desiccation tolerance, our results suggest that mechanisms of desiccation avoidance are more important than mechanisms of desiccation tolerance (Manuscript 4).

Some important mechanisms of desiccation tolerance were not measured in our study, e.g., species resistance to embolism. Previous studies found that for woody species, differences in their resistance to embolism influenced their mortality under drought and distribution (Anderegg *et al.*, 2016; Larter *et al.*, 2017; Johnson *et al.*, 2018). In grassland the quantification of species resistance to embolism is rare due to technical obstacles. By extracting data from Lens *et al.* (2016), we found it was unrelated to whole-plant drought resistance of survival or growth assessed in our study. Further, we measured pit membrane thickness, a key factor that has been suggested to influence species resistance to embolism (Li *et al.*, 2016), in six of our species with whole-plant drought resistance of survival from high to low. Consistently, no significant correlations between pit membrane thickness and whole-plant drought resistance of survival were found. In general, these results suggest that species differences in resistance to embolism may not drive differential drought resistance in grassland species. A new method

established by Brodribb *et al.* (2016) can allow to assess species resistance to embolism using an optical technique with cameras or flatbed scanners. It may contribute to enlarging the dataset in grassland species and to further testing the importance of species resistance to embolism for drought resistance.

Many coordination among traits were found in our study (Manuscript 4). These relations have been shown in disparate studies involving subsets of traits (Jackson *et al.*, 1997; Craine *et al.*, 2001; Tucker *et al.*, 2011; Zwicke *et al.*, 2015; Bartlett *et al.*, 2016; Lawson & McElwain, 2016). One of the important coordination is the mechanistic linkage between turgor loss point and leaf water potentials at stomatal closure. In angiosperms, stomatal closure is caused by the loss of turgor pressure in the guard cells that surrounding the stomatal pore, which in turn is actively regulated by abscisic acid (ABA; Raschke, 1975). McAdam and Brodribb (2016) found that turgor loss of the whole leaf triggered ABA accumulation and stomatal closure. Later work criticized their work and argued that it was a decrease in cell volume that provided endogenous signal for ABA accumulation (Sack *et al.*, 2018). Nevertheless, turgor loss is companied by a reduction in cell volume which will lead to stomatal closure (Zhang *et al.*, 2018). The positive correlation between turgor loss point and stomatal closure has been extensively tested in woody species (Brodribb *et al.*, 2003; Bartlett *et al.*, 2016). Some researchers even used turgor loss point as the values of leaf water potentials at stomatal closure when its actual value was unavailable (Martin-StPaul *et al.*, 2017). However, this relation has been rarely tested in herbaceous species. Here, we found that turgor loss point was significantly positively correlated with the leaf water potentials at stomatal closure (Manuscript 4), supporting the mechanistic linkage between turgor loss and stomatal closure. Yet, the relation between them is not 1:1, i.e., most species lost leaf turgor before stomatal closure, while a few after stomatal closure (Manuscript 4), supporting that the use turgor loss point as the proxy of stomatal closure should be treated with caution (Farrell *et al*., 2017).

Although the coordination among traits, many trade-offs and independence existed (Manuscript 4). This leaded to many combinations of traits to confer grassland species the same or different drought resistance and no main axes emerged to define a drought resistance spectrum (Manuscript 4). Consistently, focus only eight leaf and allometric traits, Valladares and Sanchez-Gomez (2006) found that in woody species, despite of the significant correlations of individual traits with species survival under drought, no single combination of traits existed to achieve high drought resistance. These results seem to challenge our capacity to establish

simple models for the predictions of drought resistance and the consequences of drought. Medeiros *et al.* (2018) even suggested to use more complicated sets of traits to increase the predictive power for predicting plants performances. However, as we found, only few traits had relatively high importance for whole-plant drought resistance (Manuscript 4). The use of these traits with high importance could largely simply our predictive models (see below).

The predictions of species drought resistance using traits

Traits, compared with whole-plant drought resistance, are relatively easy to measure and have the generality and predictivity properties (O'Brien *et al.*, 2017). Many efforts have been devoted for the 'soft' trait measurements, leading to large trait datasets (Kattge *et al.*, 2011). 'Soft traits' are the traits that are easy to measure but have indirect effects on species performances through 'hard' traits (Hodgson *et al.*, 1999). 'Hard' traits, in contrast, are relatively difficult and expensive to assess, but have mechanistic linkages with plant key processes. To our best knowledge, our traits dataset is the first comprehensive set of 'hard' traits that are important for drought resistance across multiple temperate grassland species. Some previous studies have found that 'soft' traits had lower predictive power for species distribution across moisture gradients than 'hard' traits (Tucker *et al.*, 2011; Belluau & Shipley, 2017; Belluau & Shipley, 2018). While others found that even with 'hard' traits the predictive power for species distribution was also low (Craine *et al.*, 2013; Ocheltree *et al.*, 2016). As we shown, the relation between whole-plant drought resistance and distribution was weak (Manuscript 1), and different sets of traits were important for them (Manuscript 4). Thus, the weak associations of traits with species distribution may not necessarily indicate that the traits tested were poor proxies of drought resistance.

Many studies have advocated to use turgor loss point to predict species drought resistance due to its mechanistic linkage with stomatal closure and other important hydraulic traits (Bartlett *et al.*, 2012b; Blackman, 2018; Hochberg *et al.*, 2018). In woody species, it was highly correlated with species survival under natural drought at the community level (r^2 = 0.76; Zhu *et al.*, 2017). However, in our grassland species, the explained variance of whole-plant drought resistance of survival by turgor loss point was low (20%; Manuscript 2), suggesting that other important traits are needed to improve the prediction. We extended the rapid method that assesses turgor loss point with osmotic water potential at full turgor from original woody species (Bartlett *et al.*, 2012a) to herbaceous species. The validation of this method can allow to further test the use of turgor loss point for the predictions in other herbaceous taxa.

By inclusion of important traits of drought resistance, we improved our predictive power. Only two variables, midday leaf water potential under drought and root mass in dry treatment plots, can explain 48% variance of whole-plant drought resistance of survival (Manuscript 3). Further, if forbs and grasses were treated separately, root morphological traits, i.e., root diameter, root tissue density and root specific length, can explain 56% variance in forbs, and root mass and rooting depth in irrigated treatment plots and root depth distribution 60% in grasses (Manuscript 3). These root traits can be easily assessed under well-watered conditions and thus may facilitate the prediction of grassland dynamics under climate change. The next step will be to link our models to the observed grassland dynamics that were caused by water stress at Biodiversity Exploratories (Fischer *et al.*, 2010) to assess to the predictive power of these models at community levels.

Conclusion

Assessing species drought resistance is important for predicting the consequences of drought on grassland species. Multiple processes, i.e., water uptake, water storage, water transport, water loss, membrane stability and carbon gain, are involved in promoting species drought resistance. These processes in turn entail various traits with interactions among each other. However, to my knowledge, no studies that have evaluated the relations and importance of these traits with several comparatively assessed drought performance parameters across multiple grassland species. Here using a relatively large number of species including both forbs and grasses, we answered several important questions for functional ecology and for the predictions of grassland dynamics under climate change. Although most of our species were quite resistant to drought, some of them were vulnerable. Due to the high abundance and frequency of these drought-sensitive species in German grasslands, changes in species composition and reduced productivity or other ecosystem services would be expected if no appropriate managements, such as irrigation during the growing season, were applied. Overall, our results support the use of traits for the predictions of species performances under drought. Separate models for forbs and grasses with root traits can provide relatively high predictive power. The facts that trait importance differed between forbs and grasses and between different drought performance parameters, and the contrasting mechanisms of drought resistance between herbaceous species and woody species suggest that we should take cautions with our generalizations of trait relations with performance parameters. Nevertheless, our results provide practical recommendations for trait selection and trait measurement schemes, which will aid grassland conservation and management under climate change.

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Manuscript1: Comparative drought resistance of temperate grassland species: evaluation of performance trade-offs and the relation to species distribution across moisture gradients

Comparative drought resistance of temperate grassland species: evaluation of performance trade-offs and the relation to species distribution across moisture gradients

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Abstract

Intensity and frequency of drought events are expected to increase with climate change. To improve our capability of projecting consequences of these changes for grasslands, we need a thorough understanding of species performance responses to drought, of performance trade-offs and how drought responses are related to species distributions. However, comparative and quantitative assessments of whole-plant drought resistance that allow to rigorously address these aspects are lacking for grassland species. In this study, we conducted a common garden experiment with 41 common temperate grassland species including grasses and forbs to assess species' whole-plant drought resistance as the response ratio of survival or growth in drought relative to well-irrigated conditions. Overall, survival and growth were significantly reduced under drought, with the effect varying across species. No performance trade-offs emerged between optimal growth and drought resistance of survival ('growth-stress tolerance' trade-off hypothesis), or between growth under well-watered and dry conditions ('growth rates' trade-off hypothesis). Species' moisture association was unrelated to drought resistance in terms of survival or growth, but drought sensitive species were restricted to moist habitats. Together our results indicate that drought acts as a filter excluding drought sensitive species from dry habitats, but that any trade-offs against drought resistance are weak and do not limit species distribution in moist habitats. Our results imply that additional abiotic and biotic factors play a substantial role in influencing the realized hydrological niche in grassland species. The comparative experimental assessment of species whole-plant drought resistance provides a basis to further increase our understanding of direct and indirect effects of moisture for community assembly and species distributions, and to rigorously test the importance of functional traits for drought responses. It will therefore contribute to understanding current and predicting future grassland responses to variation of moisture regimes.

Keywords

Hydrological niche; drought tolerance; forbs; grasses; response ratio; habitat moisture

Introduction

Drought is an important driver of community composition, diversity, and ecosystem function in a variety of ecosystems worldwide (e.g. Knapp et al. 2002, Engelbrecht et al. 2007a, Reichstein et al. 2013). Temperate grasslands are among the most widespread biomes on earth, exhibit high species richness, and provide important ecosystem services (Gibson 2009, Wilson et al. 2012). They periodically experience years with low rainfall that lead to drought stress (Gibson 2009). In a wide range of temperate grasslands drought decreases productivity, and influences species abundances and distribution across time and space, as well as community composition and diversity patterns (Tilman and El Haddi 1992, Buckland et al. 1997, Knapp et al. 2002, Hoover et al. 2014). In turn, the response and resilience of grasslands to drought are influences by community composition and diversity (Tilman and Downing 1994, Vogel et al. 2012, Isbell et al. 2015). With global climate change, increasing temperatures and decreasing water availability are projected during the growing season for many regions, including temperate grasslands (Orth et al. 2017, Schlaepfer et al. 2017), and the intensity and frequency of drought events is expected to increase (IPCC 2014). The importance of drought for temperate grasslands will therefore increase in the future. Species resistance to drought varies widely even within grassland communities (Buckland et al. 1997, Tucker et al. 2011, Craine et al. 2013), and this difference frequently demonstrate segregated distribution of species across moisture gradients (Silvertown et al. 2015). To appreciate the role of drought for community assembly and ecosystem function under past and current climate regimes, and to improve projections for the future, an understanding of species differential responses to drought is urgently needed.

Performance trade-offs are central in explaining species sorting along environmental gradients and species coexistence (Levins and Culver 1971, Chesson 1985, Rees et al. 2001). A prominent trade-off that has been suggested is a trade-off between growth rates under optimal conditions and tolerance against stress (i.e. ‘growth-stress tolerance’ trade-off hypothesis, Grime and Hunt 1975), which is consistent with the concepts of resource acquisition vs. conservation strategies or the slow vs. fast plant economics spectrum (Craine 2009, Reich 2014). On the other hand, a trade-off between species relative growth rate at different resource levels has been proposed to leading to species rank reversals along gradients of resource availability (‘growth rates’ trade-off hypothesis, Latham 1992). Both trade-offs have been extensively addressed, mainly with respect to light and nutrient for woody species, considering

whole-plant performance and the trait level (e.g. Sack and Grubb 2001, Kitajima and Poorter 2008, Reich 2014, but see Craine 2009 for nutrients in grassland species). In contrast, performance trade-offs with respect to drought have rarely been empirically studied despite their pervasive implications for competition, community dynamics and species distributions, especially under changing moisture regimes (e.g. Kneitel and Chase 2004). To our knowledge, for drought only two studies have explicitly tested the 'growth-stress tolerance' trade-off hypothesis at the level of whole-plant performance, both in grassland species, and both did not find a trade-off (Fernández and Reynolds 2000, Zwicke et al. 2015). The 'growth rates' trade-off has been tested in one study, to the best of our knowledge, using grassland species under high vs. low moisture conditions, and did find a strong positive correlation rather than a trade-off (Reader et al. 1993). A lack of comparative datasets on whole-plant drought performance considering both growth and survival currently hinders further testing these central hypotheses.

Associations of plant species local and regional distribution patterns with variation in soil water availability are among the most prominent biogeographic patterns (Silvertown et al. 2015 and references therein). Direct effects of water availability on plant performance as well as indirect effects through variation of the strength of biotic interactions or of other abiotic factors correlated with water availability (e.g. nutrients, light) may lead to these patterns (Normand et al. 2009, Silvertown et al. 2015). Drought periods can act as a filter, directly excluding drought-sensitive species from drier sites ('physiological tolerance hypothesis', Currie et al. 2004). On the other hand, trade-offs between drought resistance and optimal growth rates, and between performance under different moisture levels (see above), as well as trade-offs involving herbivores or pathogens, or tolerance to water logging, low nutrients or low light have been suggested to limit the occurrence of drought-resistant species under moist conditions (e.g. Baltzer and Davies 2012, Silvertown et al. 2015, Grubb 2016). This should lead to a turnover of species with increasingly lower drought resistance along moisture gradients, resulting in a negative relation between species drought resistance and their association to moist conditions. Linking species' whole-plant drought resistance, which refers to species fundamental niche regarding drought, and their association to habitat moisture allows to test to what extend species differential drought resistance in combination with soil water availability directly determines species occurrence across moisture gradients. To our knowledge, the relation of whole-plant drought resistance to species distributions has so far only been explicitly tested in tropical forests (Engelbrecht et al. 2007a, Esquivel-Muelbert et al. 2017a). Evaluation of the direct role

of drought for species distributions in grasslands is still outstanding, although it is fundamental to projecting consequences of changing moisture regimes.

To improve our understanding of species drought resistance in grasslands, several recent studies have addressed the relation of physiological and morphological traits to leaf damage under drought or to species distribution with respect to moisture or examined the coordination of traits relevant for plant drought responses (Pérez-Ramos et al. 2013, Zwicke et al. 2015, Belluau and Shipley 2017, 2018). Other studies have focused on specific traits under the assumption that they are functionally important for demographic rates and fitness (Tucker et al. 2011, Craine et al. 2013). However, our understanding of the links between traits and whole-plant responses to drought remain limited, although this link is a foundation assumption of trait-based ecology (Shipley et al. 2016). Considering whole-plant rather than leaf responses is especially important in perennial temperate grassland species, since many of them are capable of recovering even from full aboveground mortality (Volaire and Norton 2006, Zwicke et al. 2015).

Relevant comparative datasets on whole-plant performance responses to drought remain scarce and mostly restricted to woody plants (Kursar et al. 2009, O'Brien et al. 2014, Anderegg et al. 2016). The few studies that have assessed comparative species-specific survival and growth responses to drought focusing on grassland species remain confined to a small number of species (4−8 species, Fernández and Reynolds 2000, Pérez-Ramos et al. 2013, Zwicke et al. 2015, but see Reader et al. 1993 for growth responses to drought in 42 species), limiting their power to test performance trade-offs, relations to distributions and community assembly and the traits underlying drought resistance.

Given the central role of drought resistance in predicting consequences of climate change, rapidly and easily assessable proxies of species whole-plant responses to drought would be highly desirable (Bellaue and Shipley 2018). While some progress has been made towards predicting species distributions along moisture gradients in grasslands from traits, the predictive power generally remains low, especially for easily assessable and widely available 'soft traits' (Shipley et al. 2017), and/or traits are difficult and time consuming to assess, precluding comparable assessments across large species numbers (Bellaue and Shipley 2017). Wilting and drought damage can be easily visually assessed and have been used as indicators of plant drought sensitivity in horticulture, agriculture and ecological studies (IRRI 1996, Engelbrecht and Kursar 2003, Pathan et al. 2014, Belluau and Shipley 2017). Intuitively, one

might expect that species that wilt early will suffer higher mortality under drought. A strong positive correlation between visually assessed wilting and drought mortality has indeed been reported for tropical tree seedlings (Engelbrecht et al. 2007b). However, changes of leaf angle, leaf rolling and leaf abscission may also minimize water loss and extend survival under drought (Fischer and Turner 1978, Turner and Begg 1981). The relation between this promising parameter and drought survival therefore has to be established in different life forms and habitats before using it as a proxy.

In this study, we quantified whole-plant drought resistance of 41 common temperate grassland species by comparing plant performance (growth and survival) under dry and irrigated conditions in a common garden experiment. This approach allowed us to assess the importance of drought effects on species' performance, independent of effects of other abiotic (e.g. light and nutrients) and biotic factors (e.g. competition, herbivore or pest pressure; Engelbrecht and Kursar 2003). We tested the hypotheses that (1) species differ in their drought responses, (2) there are performance trade-offs with respect to drought, specifically (a) a 'growth-stress tolerance' trade-off, and (b) a trade-off between growth rates under high and low water availability, and (3) species' drought resistance is negatively related to their association to moist habitats. We additionally tested if visually assessed drought damage based on wilting and necrosis provides a suitable proxy for species whole-plant drought survival.

Materials and methods

Field site

The experiment was conducted in a meadow in the Ecological Botanical Garden (EBG) of the University of Bayreuth, Germany (49°55'19"N, 11°34'55"E). The area has a temperate climate with a mean annual temperature of 8.7°C and a mean annual precipitation of 745 mm (1998–2007, data: EBG). Mean monthly temperature ranges between -0.1°C and 17.8°C with July being the warmest month. Rainfall occurs mostly in the growing season (from May to September) with mean monthly rainfall between 60 mm and 85 mm.

Study species

We initially selected 44 focal species, 21 grasses (Poaceae) and 23 forbs (9 families including five legume species; Table S1), based on the following criteria: (a) common species in German

grasslands, based on their frequency and abundance in 150 grassland plots (4m x 4m) in Northeast, Central and Southwest Germany (Biodiversity Exploratories, Socher et al. 2012), (b) association with a wide range of soil moisture conditions (based on Ellenberg indicator values for soil water availability, M-values ranging from 3 to 7, Ellenberg et al. 1992), and (c) to include grasses and forbs including legumes and non-legumes. Grasses and forbs did not differ in soil moisture associations (median M-value = 5 for both). All species had the C3 photosynthetic pathway.

Seedlings were grown from seeds purchased from commercial seed suppliers (Rieger-Hofmann GmbH and Saaten Zeller, Germany, and Cruydt-Hoeck, Netherlands). They were germinated and grown in the greenhouse for three months (March–May, 2015) in the same substrate used in the field experiment (see below). Similar size individuals were selected within each species for the experiment to reduce effects of size variability.

Three species (*Capsella bursa-pastoris*, *Medicago lupulina*, *Trifolium repens*, all forbs) exhibited poor performance already in the greenhouse, and less than 70% survival even under irrigated conditions. We therefore excluded these three species from the analyses presented in the text. Analyses with and without these three species yielded qualitatively the same results, and analyses including the three species are presented in the supporting materials (Tables S3–S5).

Experimental design

Seedlings were transplanted to 72 plots and exposed to two treatments: a dry treatment, where irrigation was discontinued for 10 weeks in the late summer (36 plots), and an irrigated treatment, where high and favorable water availabilities were maintained throughout the experiment (36 plots). All plots were located under transparent rain-out shelters ensuring that both treatments experienced the same light and temperature conditions. One individual of each species was planted into each plot (i.e. aiming for 36 individuals per species in each treatment) in a 20 cm grid with species randomly assigned to the grid points. The distance among the plants was chosen to minimize interactions (i.e. competition or facilitation; leaves and roots were not overlapping among individuals), thus allowing us to assess the drought responses of the individual species.

Treatments were blocked to avoid cross-effects of irrigation on dry plots: two plots (1 m x 2 m) were set under each shelter (3 m x 3.5 m size, 2.1 m high at the highest point), and six shelters were blocked for a treatment (in total six blocks with 36 shelters). Plots under each shelter were set up at 0.5 m distance, and shelters and blocks had 1 m and 2 m distance to each other, respectively.

The rain-out shelters were covered with transparent plastic foil (200 μm; Gewächshausfolie UV5, folitec Agrarfolienvertriebs GmbH, Westerburg, Germany), with two sides and the lower approx. 50 cm open to allow air circulation. To expose all plants to uniform soil conditions, and to ensure that they dry down to stressfully low levels of water availability, the plots were dug out to 1 m depth and filled with sand (97% sand, 2% silt and 1% clay).

Seedlings were transplanted in the first week of June 2015, and all were regularly watered before the start of the dry treatment to allow for establishment in the soil. Irrigation was implemented with a drip-irrigation system onto the soil surface. The amount of irrigation before the start of the experiment and in the irrigated plots was adjusted individually for each plot and according to weather conditions to ensure optimal moist conditions, avoiding both superficial soil drying and waterlogging (based on inspection at least five times a week, higher irrigation on warmer/sunnier days). Irrigation was discontinued in the dry treatment plots from 3rd August to 9th October, 2015 (10 weeks, 67 days), and the soils were allowed to dry down through evapotranspiration. At the end of the drought treatment, we rewatered all plots and removed plastic covers from the rain-out shelters so that all plots were exposed to natural conditions until the next spring to allow for potential recovery from surviving below-ground parts.

The goal of the experiment was to expose all species to uniformly pronounced drought stress in order to assess drought resistance in a way that is directly comparable across all species, rather than to mimic a specific natural drought event or climate change regime. Exposing the plants to experimental drought in the field avoided common problems associated with drought experiments in pots, namely that soil water depletion is strongly influenced by plant size and differences in transpiration rates, hindering meaningful comparisons among species (Comita and Engelbrecht 2014).

Fertilizer (Terra Plus N 12+4+6, 30 g m^{-2}) was applied twice before the start of the drought treatments to minimize potential nutrient limitation. Plots were regularly weeded and

surrounding areas were mowed to avoid competition from non-target species. All plots were fenced to avoid mammalian herbivores such as dears or hares.

Environmental conditions

In the study area, 32 days or 42 consecutive days without rain during the growing season are considered extreme meteorological drought events based on the 100-year and 1000-year recurrence, respectively (Jentsch et al. 2011). The 2015 summer was additionally extremely hot and dry with the lowest rainfall since 1901 in Central Europe (Orth et al. 2017), which was also reflected in the local conditions during the experiment (Table S2). The naturally dry, sunny and warm conditions ensured high evapotranspiration rates so that soils in the non-irrigated plots under the rainout-shelters dried out fast and strongly, and plants clearly experienced drought stress (see below).

We monitored soil water status with gypsum blocks (GB-1 and KS-D1, Delmhorst, NJ), installed at 15 cm soil depth in every plot and additionally at 30 cm depth in six haphazardly chosen plots in each treatment. Readings were initially taken daily after the start of the treatments, and later every three to five days around midday. Meter readings were converted to soil water potentials according to the device instructions. In the irrigated plots soil water potentials remained above -0.04 MPa throughout the experiment. In the dry treatment, water potential readings declined sharply after discontinuation of irrigation. After 18 days the first dry plot reached values below -1.5 MPa, exceeding the lower measurement range of the equipment and the permanent wilting point in agricultural plants (Veihmeyer and Hendrickson 1928). After 50 days all dry plots had reached values below -1.5 MPa.

Air temperature and relative humidity were measured hourly in three randomly selected plots in each treatment at 1.8 m height during the experiment (i-buttons, DS1920, Maxim Integrated, CA). Light was repeatedly measured inside and outside the rain-out shelters with the built-in light sensor of a leaf porometer (AP4, Delta-T, Cambridge) to calculate light transmittance. Daily mean air temperature was 19.4°C, daily mean relative humidity 74.7% and light transmittance 86 ± 3%, and values did not differ between the treatments ($p > 0.3$ for all).

Assessments of plant performance and drought sensitivity

We assessed species performance based on three parameters: visual drought-damage, survival and relative growth rates (RGR). Visually observable drought damage in each individual was scored weekly based on wilting and necrosis in the dry plots (starting in week 2). Categories (1−9) were modified from existing systems for visual evaluation of drought damage in rice and tropical seedlings (IRRI 1996, Engelbrecht and Kursar 2003; Table 1).

Survival was assessed based on above-ground organs (leaves and stems) at the end of the dry treatment, and additional checks for resprouting from surviving below-ground organs in the next growing season (June 2016) in all of the dry and irrigated plots. We quantified species' survival in the irrigated and dry treatment ($S_{irrigated}$ and S_{dry}) as the percentage of individuals that survived in the respective treatment relative to the initial number of individuals. Growth was assessed in 6 plots randomly selected for each treatment. We monitored growth non-destructively based on the increase or loss of the plants' projected green leaf area (LA, compare Breitschwerdt et al. 2018) to allow repeated monitoring of growth and survival on the same individuals. LA was determined as the area of an octagon with the focal plant in the center, and with the endpoints of living leaf area along eight plant radii (in 45° angles) representing the corners. LA was assessed twice, in the first week and sixth week of the treatment, for all individuals in a subsample of six plots for each treatment. We calculated RGR ($cm^2\ cm^{-2}\ day^{-1}$) from the consecutive measurements in each individual as $RGR = (LA_2 - LA_1)(LA_1)^{-1}(T_2 - T_1)^{-1}$ (Hunt 1978), where LA_1 and LA_2 are projected green leaf area at time T_1 and time T_2. Species RGRs under irrigated conditions (combined with high light and nutrients, for survivors, see below) were marginally significantly correlated with comparative assessments of optimal growth rates (RGR_{max}) of subset of our species in the literature ($p < 0.1$ for each individual study: 24, 9 or 13 overlapping species from Grime and Hunt 1975, Poorter and Remkes 1990, Ryser and Wahl 2001, respectively), supporting that the method usefully captured comparative growth rates.

Species RGR was assessed both based on survivors only and based on all individuals including dead ones. Growth of survivors focuses on those individuals that will contribute to future population dynamics and is most frequently assessed. However, this parameter does not capture the loss of leaf area (or biomass) occurring in the plants that died, which is relevant for a community or ecosystem perspective. These two aspects of growth do not necessarily correlate (Engelbrecht and Kursar 2003).

A specific focus of our study was to assess the species' comparative whole-plant drought resistance. We calculated species' drought resistance (DR) as the response ratio of survival and growth in dry relative to irrigated plots (compare Engelbrecht and Kursar 2003) to present intuitive and consistent indices for both performance parameters, i.e. $DR_{survival} = S_{dry}/S_{irrigated}$, where S_{dry} and $S_{irrigated}$ are the % of individuals of a species that survived in the dry and the irrigated treatment, respectively, and $DR_{growth} = RGR_{dry}/RGR_{irrigated}$, where RGR_{dry} and $RGR_{irrigated}$ are the median RGR in each treatment.

Statistical analyses

To test the effects of treatment, species and their interactions (treatment x species) on survival and growth, generalized linear mixed effects models (GLMM) with binomial distribution and linear mixed models (LMM) were fitted for survival and growth data, respectively. Treatment and species were used as fixed effects and blocks and plots nested in blocks were used as random effects in each model. Significance of the random effects was assessed by comparing the log-likelihoods of models with and without the random effects. All models were fitted over all species as well as separately for grasses and forbs. For RGR, we fitted two different models considering only surviving individuals or all individuals including dead ones (see above). Using separate models for each species, we additionally assessed the significance of the effect of the drought treatment on survival and growth of each individual species. For every model, treatment was used as a fixed effect and block as a random effect. Drought resistance ($DR_{survival}$ and DR_{growth}) was compared between grasses and forbs using a t-test.

To test the relations between growth under optimal conditions and drought resistance of survival ($RGR_{irrigated}$ vs. $DR_{survival}$, 'growth-stress tolerance' trade-off hypothesis), between species' growth under irrigated and dry conditions ($RGR_{irrigated}$ vs. RGR_{dry}, 'growth rates' trade-off hypothesis), and between species' drought resistance with respect to survival and growth ($DR_{survival}$ vs. DR_{growth}), we used Pearson correlations and again conducted analyses over all species and separately for each life form. $RGR_{irrigated,}$ measured under irrigated and concurrently high light and nutrient conditions, was used as growth rate under optimal conditions to test the 'growth-stress tolerance' trade-off.

Ellenberg indicator values (Ellenberg et al. 1992) categorize species based on non-systematic observations of their abundance along environmental gradients in Germany. They have been shown to be highly correlated with quantitative assessments of associations with habitat

environmental conditions across Europe and can thus be considered reliable indicators of species association with environmental factors (Schaffers and Sýkora 2000, Diekmann 2003). We used Ellenberg indicator values for moisture (M-values, Table S1) as an index of species moisture association. Previous studies have shown that Ellenberg indicator values can be treated as continuous values in statistical analysis (Ellenberg et al. 1992, Diekmann 2003, Bartelheimer and Poschlod 2016). We thus analyzed the relations of $DR_{survival}$ and DR_{growth} to M-values using regression analysis. Species that showed no association with moisture regimes (M-value = X) were excluded from these regression analyses. To test if species that are found over a wide range of moisture conditions have a higher drought resistance than species associated to distinct moisture regimes (Currie et al. 2004), we additionally compared drought resistance ($DR_{survival}$ and DR_{growth}) between species with an M-value of X (no association) and species with assigned M-values using a t-test.

To evaluate if visually assessed drought damage after a relatively short period is a useful proxy to predict longer-term survival under dry condition, we related % survival after the 10-week intense drought to the percentage of severely drought-damaged individuals of each species (drought-damage category 5–9, see Table 1) after various periods of drought. Additionally, the significance levels were evaluated at $\alpha = 0.05$ with Bonferroi correction (Rice 1989). However, the results and discussion are based on unadjusted values, as we were mainly interested in a relation at a single period.

To test if species ranking of drought survival depends on the duration/intensity of drought, we examined correlations between above-ground survivals (% individuals in drought-damage category 9) after different lengths of drought treatment, and with the final survival (S_{dry}).

We visually evaluated normality of the residuals in all analyses. DR_{growth} was consequently log transformed to improve normality, and for handling negative values in DR_{growth} 4 was added as a constant value to the data prior to applying the log transformation.

Data were analyzed in R version 3.3.3 (<www.r-project.org>) using the lme4 (Bates et al. 2017), lsmeans (Lenth 2017), and car (Fox and Weisberg 2011) packages.

Table 1 Drought-damage categories based visual assessments. The categories were modified from systems for rice (IRRI 1996) and for tropical tree seedlings (Engelbrecht and Kursar 2003).

Category	Visual characteristics
1	no signs of drought stress
2	slight signs of wilting (leaf angle changes, rolling, or folding) without leaf necrosis
3	strong signs of wilting (leaf angle changes, rolling, or folding) without leaf necrosis
4	slight leaf tip drying (necrosis)
5	tip drying (necrosis) extended to ¼ length in up to 25% of the leaves (normally old leaves)
6	tip drying (necrosis) extended to ¼ length in up to 50% of the leaves (or 25% of leaves fully dried)
7	more than 50% of the leaves fully dried
8	more than 70% of the leaves fully dried
9	all above-ground parts dead

Results

Drought damage and resprouting

All species exhibited at least some visual drought damage in the dry treatment, and its progression varied strongly among species (Fig. 1, Fig. S1). Many species maintained living aboveground biomass throughout the intense drought treatment, some with pronounced wilting and tissue necrosis (e.g. the grasses Holcus lanatus and Lolium perenne), while others showed wilting but only slight necrosis (e.g. the forbs Achillea millefolium and Leucanthemum vulgare). For a few species, tissue death started early (week 1) and all aboveground biomass had died after the 10 weeks of drought in more than half of the individuals (e.g. the grass Poa trivialis and the forb Cerastium holosteoides). In general, grasses exhibited leaf rolling and folding early (week 1 and 2), and already showed necrosis in week 2 of the drought treatment. In comparison, in forbs leaf angle changes and/or leaf shrinking occurred later and leaf necrosis progressed slowly (only the three Ranunclus species and Cerastium holosteoides showed fast necrosis). In the irrigated treatment, no drought damage was observed.

In many species resprouting occurred, i.e. individuals without any living aboveground biomass after the drought period resprouted from surviving belowground organs. 83% of the species exhibited at least some resprouting (of the 36 species where some individuals exhibited

complete aboveground mortality). Within species, up to 40% of the individuals without living aboveground biomass directly after the drought resprouted (see Table 1S for the species and number of the individuals that resprouted). Overall low mortality (see below) did not allow for a meaningful formal analysis of species' resprouting capacity, which was not the focus of this study.

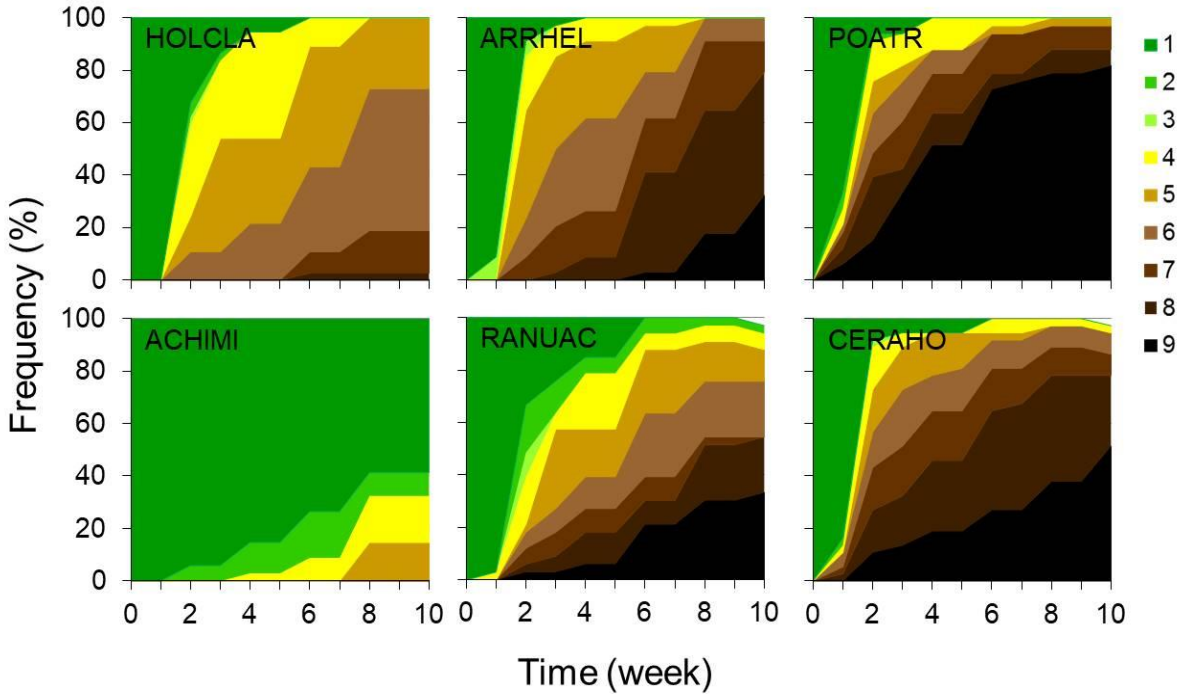

Figure 1 Progression of visual damage in selected temperate grassland species over 10 weeks of drought as examples. The color scale represents damage categories from no visual sign of drought stress (1, dark green) through progressive signs of wilting or rolling, and tissue necrosis to complete death of all above ground plant parts (9, black). For a description of the drought damage categories and for species codes, see Table 1 and Table S1, respectively. Grasses are presented in the first row, and forbs in the second row. Shown are two examples for species with low mortality and either with early signs of drought damage (HOLCLA) or few and late visual signs of stress (ACHIMI), two examples of species with high mortality and early visual drought damage (POATR, CERAHO), and two examples for intermediate species with moderate mortality and early visual drought damage (ARRHEL, RANUAC). See Figure S1 for graphs for all species.

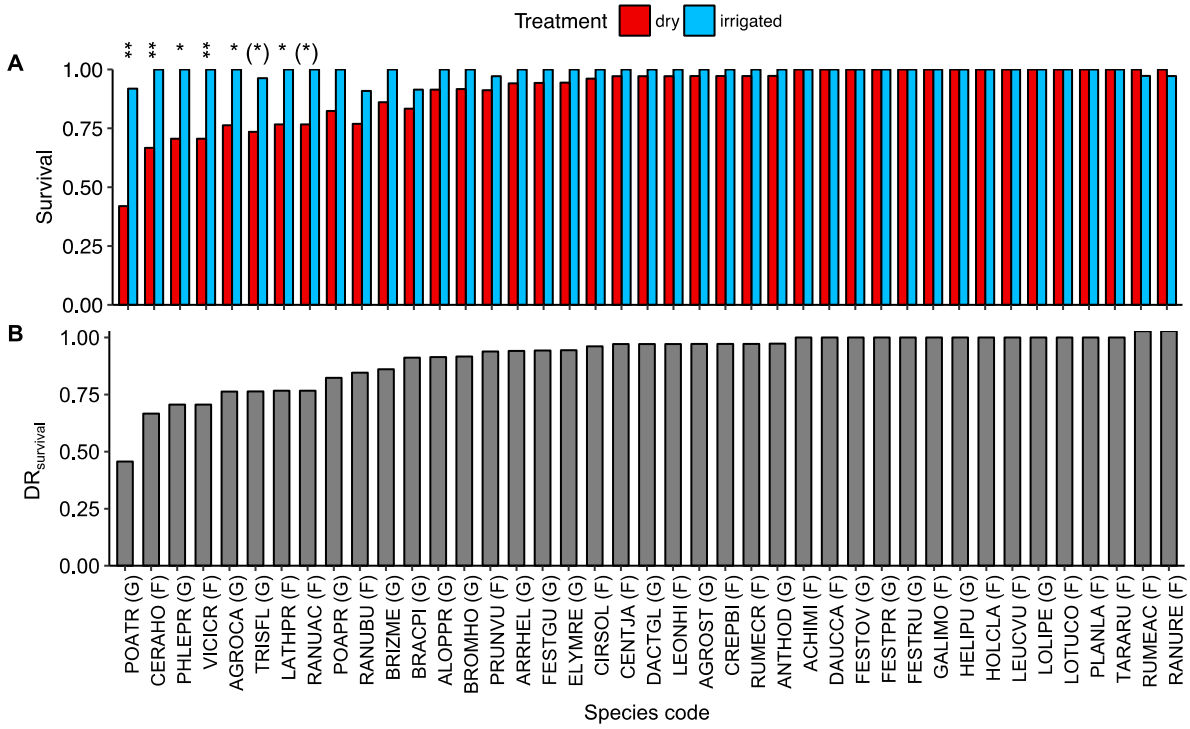

Figure 2 (A) Survival of 41 temperate grassland species under dry and irrigated conditions (red and blue bars, respectively). Given are the proportion individuals that survived relative to the initial number of individuals. Significance of treatment effects on survival within each species is given above the bars ((*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$), for details see Tables S3 and S4. **(B)** Drought resistance of survival ($DR_{survival}$, response ratio of survival in the dry treatment relative to the irrigated treatment). Species are sorted by increasing drought resistance. For species codes, see Table S1. Grasses and forbs are indicated with G and F, respectively.

Drought effect on survival and growth

Survival was overall high. In all 41 species analyzed, survival in the irrigated treatment was above 90% and even under the intense dry treatment 29 of the species (70%) exhibited more than 90% survival (Fig. 2A). The dry treatment had a significant negative effect on survival of grasses, but no effect on forbs, resulting in a marginally significant effect over all species (GLMM; Table 2). Species significantly differed in their survival (overall and within grasses or forbs), and there was no significant treatment x species interaction (Table 2). In six of the individual species (15%), the dry treatment had a significant negative effect on survival ($p < 0.05$, GLMM) and in further two species a marginally significant effect ($p < 0.1$), while the remaining the species (78%) showed no significant treatment effect (Fig. 2A, Table S4).

Aboveground survival (i.e. % of individuals in drought-damage category 9) after different periods of drought (from 2 to 10 weeks) was highly positively correlated with each other and with the final survival (S_{dry}) ($r > |0.8|$, $p < 0.001$ for all), indicating that species ranking of drought survival did not change with progressing length of drought.

Whole-plant drought resistance based on survival ($DR_{survival}$, i.e. survival under dry relative to irrigated conditions, the response ratio), varied continuously across the species (Fig. 2B), and did not differ between grasses and forbs ($p > 0.05$, t-test). The most drought-sensitive species was *Poa trivialis* (grass) followed by *Cerastium holosteoides* (forb).

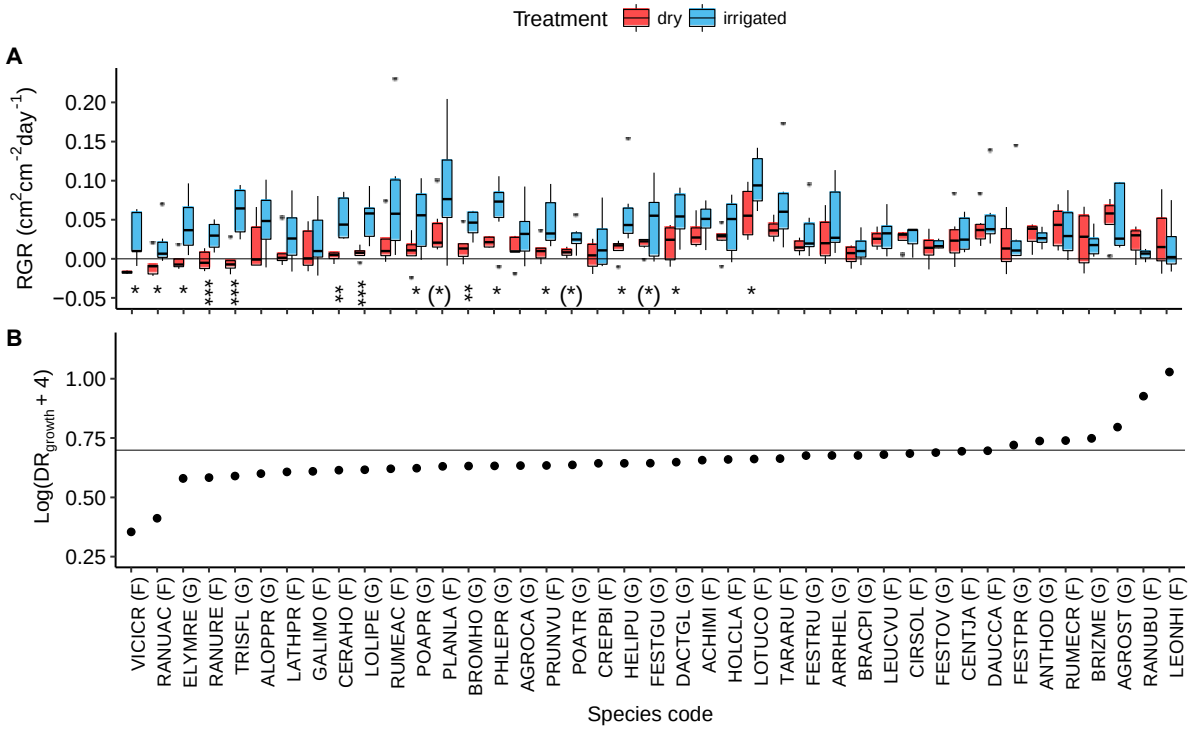

Figure 3(A) Relative growth rate (RGR) of projected living leaf area for 41 forb and grass species under dry and irrigated conditions (red and blue boxes, respectively). Boxes give the median, the 25% and 75% quartiles. Significant treatment effects within each species are given below the box plots ((*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), for details see Tables S3 and S4. RGR is based on surviving individuals. For results based on all individuals including dead ones see Fig. S2. **(B)** Drought resistance of growth (DR_{growth}, ratio of median RGR in the dry relative to the irrigated treatment). Note that the data are log-transformed. Values below the line at 0.7 indicate a decrease in RGR in the dry relative to the irrigated treatment. Species are sorted by increasing drought resistance. Grasses and forbs are indicated as G and F, respectively. For species codes and sample sizes, see Table S1.

Table 2 Effects of drought treatment and species on survival and growth. Results were from generalized linear mixed models (GLMM, for survival) and linear mixed models (LMM, for growth of survivors, RGR) with treatment, species and treatment x species interaction as fixed effects and block and plot as random effects. Models were set up for over all 41 species and for the two life forms (grasses and forbs) separately. For fixed effects Chi^2 (X^2) and F-values are given for survival and for growth, respectively, and for random effects X^2 are given. Significance levels are presented as (*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. For the results for 44 species and for analyses of growth for all individuals including dead ones, see Table S3.

	Treatment	**Species**	**Treatment x species**	**Block and plot**
Survival				
All	20.41(*)	106.148***	13	40.58***
Grasses	17.09*	71.35***	11.6	33.37***
Forbs	3.54	34.92*	1.5	3.33
RGR				
All	7.98*	2.69***	1.08	25.48***
Grasses	12.05*	1.11	1.19	6.07*
Forbs	4.49	4.09***	1.02	13.38**

Relative growth rate (RGR) was also significantly lower in the dry than in the irrigated treatment across all species and for grasses, but not for forbs (LMM; Fig. 3A, Table 2). Species differed significantly in RGR among all species and forbs (but not among grasses) with no treatment x species interaction (Table 2). Under irrigated conditions, RGR was positive for all species. Even under dry conditions, surviving individuals of most species maintained positive RGR, and net losses of projected leaf area were only found in five of the species (12%). Within species, the dry treatment had a significant negative effect on RGR of the surviving individuals in 14 of the species (34%) and a marginally significant effect in three species (LMM; Fig. 3A, Table S4), whereas the remaining 59% showed no treatment effect on RGR. Based on all individuals (i.e. including dead ones) the negative effect on growth was significant in 16 of the species (39%) and a marginally significant effect in four species (Fig. S2, Table S4).

Whole-plant drought resistance based on growth (DR_{growth}) again varied continuously across species (Fig. 3B), and did not differ between life forms ($p > 0.05$, t-test).

DR_{growth} considering only surviving individuals or all individuals including dead ones were highly significantly correlated across all species as well as within life forms ($p < 0.001$ for all,

Table S5), and results of all analyses were qualitatively similar (Fig. S2, Table S3). All following analyses presented in the text refer to survivors only.

Performance relations

We found no indication of a trade-off between optimal growth and drought survival. Growth in the irrigated treatment ($RGR_{irrigated}$) and drought resistance ($DR_{survival}$) were unrelated across all species and within grasses, and marginally positively related within forbs (Fig. 4B, Table S5). $DR_{survival}$ was also unrelated to RGR_{max} from the literature (24, 9, 13 species from Grime and Hunt 1975, Poorter and Remkes 1990, Ryser and Wahl 2001, respectively, $p > 0.1$ for species sets from all individual studies), further supporting our results.

Species growth rates in the irrigated and the dry treatment ($RGR_{irrigated}$ vs. RGR_{dry}) were marginally positively correlated over all species, with a significant positive relation within forbs and, no relation within grasses (Fig. 4A, Table S5), indicating that there was clearly no trade-off between growth rates under high and low moisture conditions.

The drought resistance of growth and survival (DR_{growth} and $DR_{survival}$) were again marginally significantly positively correlated with each other across all species and within forbs (Fig. 4C, Table S5), i.e. growth reduction under drought did not lead to improved drought resistance in terms of survival in our species.

Relations of drought resistance and habitat moisture associations

Species habitat association with moisture (Ellenberg's moisture values, M-value) was not related to drought resistance ($DR_{survival}$ and DR_{growth}) over all species nor within life forms ($p > 0.05$; Fig. 5A and B). However, the four most drought-sensitive species ($DR_{survival} < 0.8$) were restricted to moist habitats (M-value ≥ 5, Fig. 5A, lower right corner), while more drought-resistant species ($DR_{survival} > 0.8$), exhibited a large range of moisture associations (M-value from 3 to 7, Fig. 5A). The 11 species indifferent to habitat moisture (M-valaue = X) exhibited a wide range of $DR_{survival}$ and DR_{growth}. Species with narrow ranges with respect to moisture (assigned M-values) did not have a lower drought resistance than species with a wide range (M-value = X) for both $DR_{survival}$ and DR_{growth} ($p > 0.05$ for both, t-test).

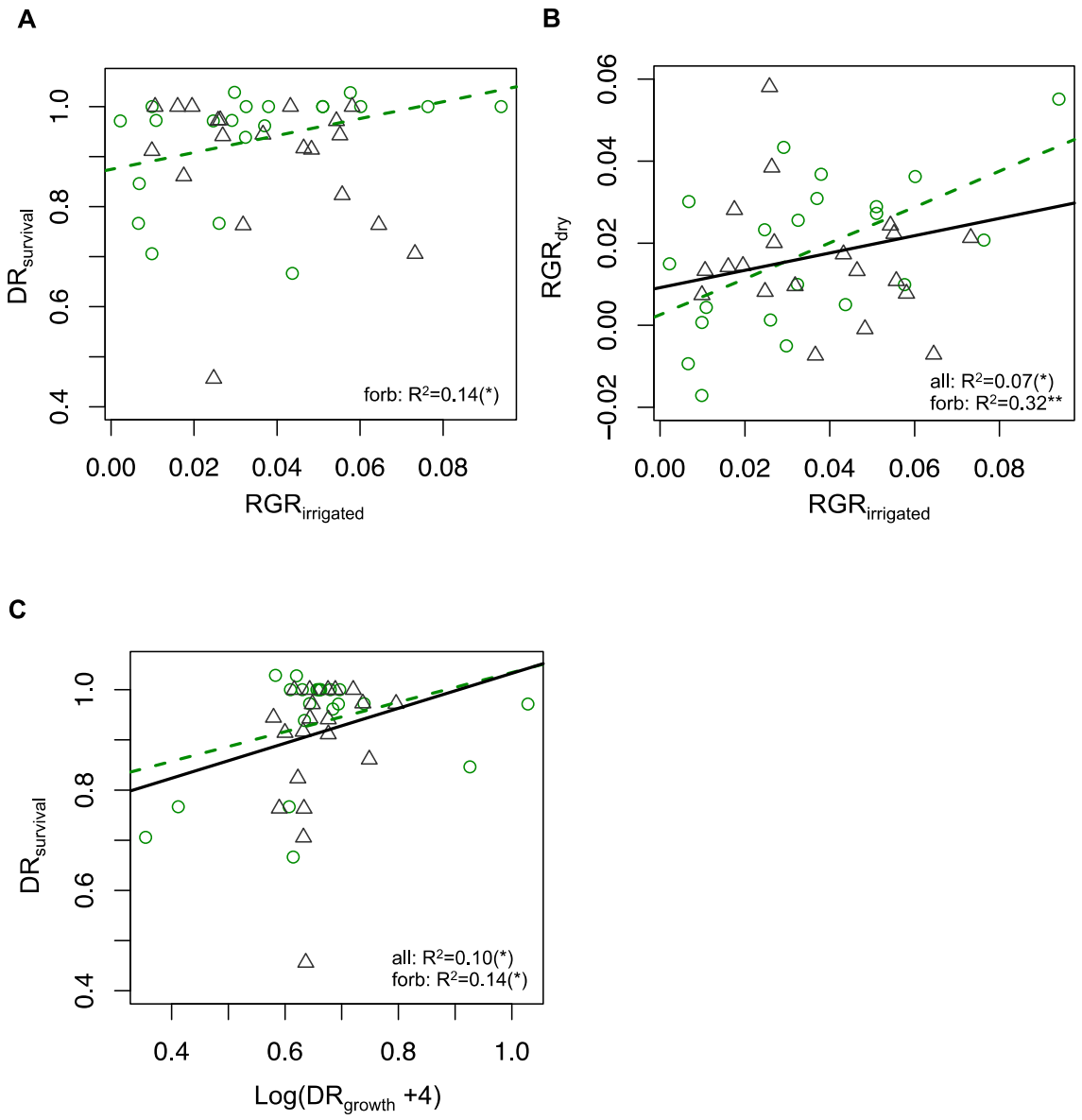

Figure 4 Performance relations with respect to drought across 41 forb and grass species. Relations **(A)** between growth under irrigated conditions and survival ($RGR_{irrigated}$ vs. $DR_{survival}$; test of 'growth-stress tolerance' trade-off hypothesis), **(B)** between growth under irrigated and under dry conditions ($RGR_{irrigated}$ vs. RGR_{dry}; test of 'growth rates' trade-off hypothesis), and **(C)** between drought resistance of growth and survival (DR_{growth} vs. $DR_{survival}$). Grasses and forbs are indicated as gray open triangles and green open circles, respectively. Species median RGR are given, and DR_{growth} was log transformed (compare Fig. 3). Significant correlations over all species are given as black solid lines, for forbs as green dashed lines (no significant relations for grasses). R^2 and the significance level ((*) $p < 0.1$, ** $p < 0.01$) are given for significant correlations (only).

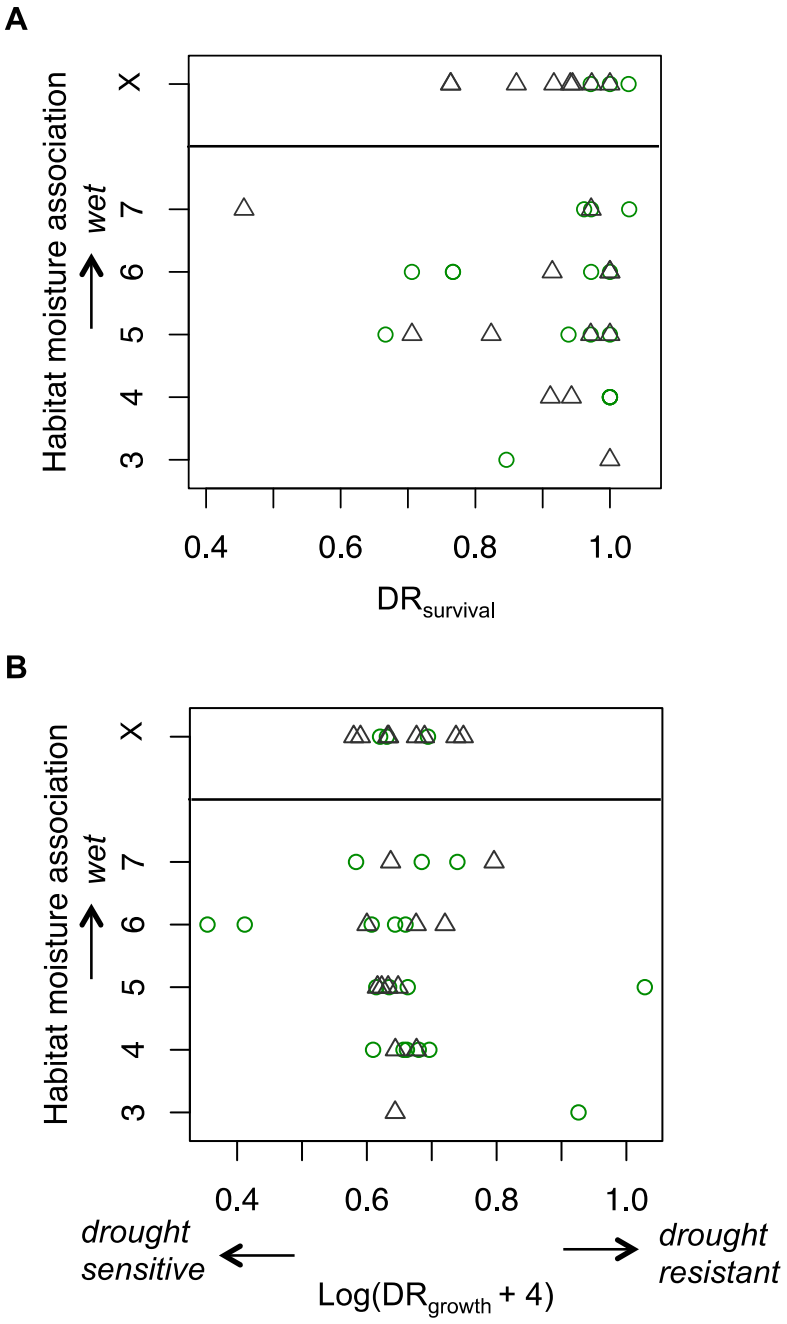

Figure 5 Relations of species' drought resistance with respect to **(A)** survival ($DR_{survival}$) and **(B)** growth (DR_{growth}) to their habitat associations with moisture. Species associations with moisture are given as Ellenberg's indicator values for moisture (M-value, Ellenberg et al. 1991), with low values indicating association with dry habitats. 'X' indicates species that are indifferent to habitat moisture, and were not included in the regression analyses. Grasses are indicated as gray open triangles and forbs as green open circles. Note in (A) that none of the drought sensitive species ($DR_{survival} < 0.8$) was associated to dry habitats (M-value < 5, lower left corner), while several drought resistant species were associated with moist habitats (upper right corner).

Relation between visual drought damage and drought survival

Survival after the 10 weeks of drought (S_{dry}) was significantly positively related to the percentage of individuals that exhibited severe visual drought damage (i.e. drought-damage categories 6−9). The relation already emerged in the second week of the dry treatment and remained highly significant within life forms and over all species throughout the experiment (Fig. 6, Table S6). The highest R^2 emerged in the third and fourth week of drought (i.e. R^2= 0.4–0.5 over all species and within grasses or forbs). Similar relations emerged between drought resistance of survival ($DR_{survival}$) and wilting (Table S6).

For drought resistance of growth (DR_{growth}), the relations were overall much weaker and not consistently significant through time over all species and within forbs, and no significant relation emerged in grasses, for both surviving individuals only and all individuals including dead ones (Table S6).

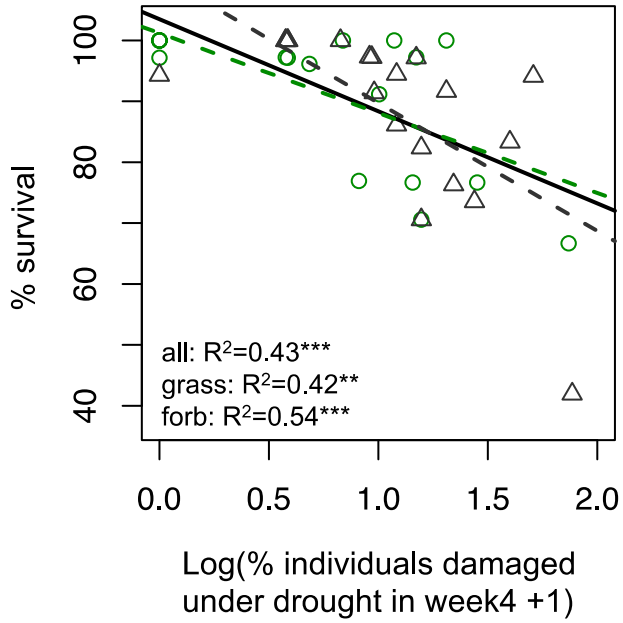

Figure 6 Relation between the % individuals that showed visual signs of severe drought damage (drought-damage categories 5−9, see Table 2) under drought in week 4 and the % survival after intensive drought across 41 species. The relations over all species, grasses, and forbs were all significant (black solid line, gray and green dashed lines, respectively). The respective R^2 and the significance level (** $p < 0.01$, *** $p < 0.001$) are given. For relations after different time periods and different species sets, see Table S6. Grasses are given as gray open triangles and forbs as green open circles.

Discussion

Drought resistance of temperate grassland species

Drought had an overall negative impact on survival and growth of common temperate grassland species, and drought damage increased with drought duration. However, more than 70% of the species survived well (> 90% survival) over the experimental drought, indicating that many species in temperate grasslands are well adapted to even intense drought conditions. These findings are consistent with previous studies, which reported reduced biomass productivity during drought, but high resilience after the drought for natural and experimental temperate grassland communities (Grime et al. 2008, Kreyling et al. 2008, Gilgen and Buchmann 2009, Vogel et al. 2012, Hoover et al. 2014, Hofer et al. 2016). Nevertheless, species responded differentially to drought, both in terms of survival and growth. Differential responses of species to drought have been suggested to alter species distribution, and the composition and diversity of grassland communities (Silvertown et al. 1999, Grime et al. 2000, Hoover et al. 2014). To our knowledge this is the most extensive study that directly and experimentally assessed comparative whole-plant drought responses of individual species in grasslands. It allows for the first time to rigorously test trade-offs and relations between performance parameters with respect to drought, and the relation of individual species drought responses to distributions and community composition of grasslands across natural gradients of soil moisture. It also provides an urgently needed basis for testing the relative importance of various traits and their combinations for drought resistance of grassland species.

Our species ranking held under a wide range of drought intensities, as indicated by the strong correlations of species survival after various lengths of experimental drought. The actual performance of the species in our drought experiment should, however, not be interpreted as directly representing performance of grassland plants under natural extreme drought events of comparable length, because the soil water status as well as the soil-atmosphere water potential gradient that plants experience are highly dependent on actual weather conditions as well as soil characteristics and site hydrology.

Quantification of plant drought resistance

Our experimental approach allowed to comparatively quantify effects of drought conditions on individual species and to separate them from effects of non-drought related factors acting on

the plants (Engelbrecht and Kursar 2003, Engelbrecht et al. 2005). Across treatments soil water potential varied, while other environmental abiotic and biotic factors that influence plant performance remained constant, so that all differences between treatments were either a direct consequence of low water availability for the plants, or indirect consequences, e.g. through altered soil and plant nutrient relations, or plant defenses to herbivore or pest (Engelbrecht et al. 2005, Jactel et al. 2012, Delgado-Baquerizo et al. 2013). Effects of competition and facilitation were excluded by avoiding any overlap of roots or leaves, allowing to assess species-specific effects of drought. At the same time, all species were exposed to the same soil-atmosphere water potential gradient, i.e. all species were exposed to the same rate of drying leading to severe drought, and the same horizontal soil water gradients and air humidity. The 'innate whole-plant performance response' of the individual species, integrating the full suite of molecular, physiological and morphological traits and their plasticity, could therefore be compared across species. The species resistance to drought, i.e. to low levels of water availability, represents the low resource side of the fundamental hydrological niche of a species.

In contrast to our experimental approach, complex interactions between abiotic and biotic factors, and changes of such interactions across resource gradients preclude rigorously isolating drought effects and comparing them across individual species based on observational data across spatial or temporal moisture gradients, or on rainfall manipulations in natural or experimental plant communities (Beier et al. 2012, Condit et al. 2013). On the other hand, in pot experiments, useful species comparisons are defied by the differential drawdown of soil water in plants with different transpiration rates due to size and/or species-specific stomatal responses, and restrictions of root development by pot size, unless careful precautions are taken (Poorter et al. 2012, Comita and Engelbrecht 2014). Few studies have therefore usefully assessed comparative species-specific responses to drought in terms of survival and growth (Engelbrecht et al. 2007a), and in herbaceous plants these remained restricted to few species (less than 8 species; Fernández and Reynolds 2000, Pérez-Ramos et al. 2013, Zwicke et al. 2015).

The fact that experimentally quantifying comparative species drought resistance based on whole plat performance responses requires tremendous effort in terms of time and labor motivated the use relatively easily measured traits to characterize species' drought resistance, under the assumption that they are suitable proxies. For example, leaf water potential at

stomatal closure has been characterized as 'physiological drought tolerance' across grass species (Craine et al. 2013), and some studies have linked this proxy to other traits, which are hypothesized to shape drought resistance (Tucker et al. 2011, Farrell et al. 2017). However, the importance of this and other traits for drought performance of herbaceous species remains poorly established. Other studies have linked traits to species distributions across habitat or biome moisture gradients (Tucker et al. 2011, Bartlett et al. 2012, Belluau and Shipley et al. 2017, Shipley et al. 2017). Nevertheless, the predictive power of these relations was low, and the physiological mechanisms and/or the ecological processes leading to these links remain unresolved, especially as other factors than moisture itself additionally influence species distributions (e.g. Condit et al. 2013, Silvertown et al. 2015). Establishing links between traits and performance responses to enable predicting ecological phenomena across species and environments is a main objective of trait-based community ecology (Shipley et al. 2016). Comparative whole-plant performance response to drought can provide the necessary link to test the importance of traits and trait combinations for species drought responses, and to understand how these affect community composition and species distributions across moisture gradients, pertaining to their realized hydrological niche. Characterization of species' fundamental niche (species drought resistance) is an important step to understand species realized niche in the real, complex world with confounding factors and multispecies interactions (Poorter et al. 2016).

Visual drought damage - a useful simple proxy for whole-plant drought resistance?

Simple proxies for species resistance to drought are urgently needed to expand evaluations of its role for community composition and diversity, and for species distributions under current and future conditions to many more species. In our study, the percentage of plants exhibiting severe visual drought damage after a short duration of drought (2−4 weeks) was strongly correlated with species differential survival of the 10-week drought (Fig. 6, Table S6). Similar results have previously been found for temperate grassland species (Zwicke et al. 2015) and for seedlings of tropical woody species (Engelbrecht et al. 2007b). Our results confirm for grassland species that simple, fast and inexpensive visual assessments of the percentage of wilted or damaged individuals can provide a suitable ranking of species survival for longer and more severe drought of different length and intensity.

Performance trade-offs with respect to drought?

We found no indication for a trade-off between relative growth rates in the irrigated treatment and drought resistance in terms of survival ('growth-stress tolerance' trade-off, Fig. 4A), nor for a trade-off between relative growth rates in the irrigated and dry treatment ('growth rates' trade-off, Fig. 4B), despite of a large (> 5-fold) variation in growth rates of well-watered plants across species. Any trends we found were even in the opposite direction.

Although the lack of trade-offs was unexpected, it was consistent with previous studies that also found no support for the 'growth-stress tolerance' trade-off for grassland species at the level of whole plant performance (Fernández and Reynolds 2000, Zwicke et al. 2015). Indirect evidence for the 'growth-stress tolerance' trade-off has been provided by relating species moisture association with growth rate in grassland species (Bartelheimer and Poschlod 2016) and tropical woody seedlings (Gaviria et al. 2017). At the trait level, evidence for a trade-off between some traits enabling fast-growth and proxies of drought resistance was also reported for grassland species (Craine et al. 2013) and trees (Reich et al. 2013). However, plants cope with and adapt to drought through various different processes such as minimizing water loss, maximizing water uptake and transport, and maintaining carbon gain (Choat et al. 2018). Morphological, anatomical and physiological traits relevant for these processes can be coordinated among each other or trade-off, but can also be orthogonal to each other (Tucker et al. 2011, Zwicke et al. 2015). In addition, many relevant traits exhibit phenotypic plasticity in responding to varying soil moisture (Jung et al. 2014, De Vries et al. 2016). The many physiological processes involved in growth and drought resistance, and the complexities in trait relations and plasticity, may lead to independent variation of species whole-plant drought resistance and maximum growth rate.

Adaptations to high resource conditions have been hypothesized to preclude optimal trait combinations under low resource conditions, leading to rank reversals across resource gradients (Latham 1992), such as the hypothesized 'growth rates' trade-off. In contrast, we found that species growth rates in the dry and irrigated treatments were independent in grasses, and even significantly positive in forbs and marginally across all species. The studies testing the hypothesis in grassland species found a rank reversal under nutrient rich vs. poor conditions, but not under different water and light conditions (Reader et al. 1993, Meziane and Shipley 1999), and in woody species with respect to drought or to light (Dalling et al. 2004, Baraloto et al. 2006, Kitajima and Poorter 2008) and in shoreline species regarding nutrients

(Keddy et al. 2000) overall also found no evidence of consistent rank reversals of growth rate across resource levels.

The lack of a 'growth-stress resistance' or 'growth-rates' trade-off with respect to drought implies that these performance trade-offs are not dominant drivers of hydrological niche differentiation or maintenance of diversity in temperate grasslands. Other factors, such as nutrients or pest pressure, and/or other coexistence mechanisms such as temporal fluctuations in limiting factors (i.e. storage effect), competition-defense trade-offs or negative density dependence may be more important for community assembly and species distributions across moisture gradients in temperate grasslands (Harpole and Tilman 2007, Chesson and Kuang 2008, Adler et al. 2013).

Relation between survival and growth under drought

Drought resistance in terms of survival and of growth rate of the surviving individuals ($DR_{survival}$ vs. DR_{growth}) were marginally positively related to each other across all species and within forbs, and unrelated in grasses (Fig. 4C) in our study. The clear lack of a negative relation indicates that reductions in shoot growth did not contribute to species' survival by minimizing transpirational leaf area through leaf deciduousness. Our findings for temperate grassland species differ from results on Mediterranean grass species (Volaire et al. 1998, Pérez-Ramos et al. 2013). Such differences imply that strategies of drought resistance vary across ecosystems, and underline the importance of testing relations of traits to whole-plant drought performance in different systems and/or species with different life histories.

When including dead individuals for growth, which contributing to total decrease of productivity in community level, giving better insight for a decrease in productivity the relations were significantly positive across all species as well as within both life forms (Table S5).

Drought resistance and species distribution

Species differential drought resistance is expected to shape species distribution across moisture gradients (Silvertown et al. 1999, Hoover et al. 2014). Relations between physiological traits related to water relations and gas exchange and species' habitat wetness support this expectation (Belluau and Shipley 2017). However, contrary to our expectations, we found that

species whole-plant drought resistance of survival or growth ($DR_{survival}$ or DR_{growth}) was not related to habitat associations with moisture (Fig. 5). Nevertheless, the most drought-sensitive species in our study were restricted to moist habitats, consistent with drought acting as a filter that excludes drought-sensitive species from dry habitats. At the same time, drought resistant species were associated to a wide range of moisture conditions, which in turn is consistent with the observed lack of 'growth-drought tolerance' or 'growth rates' trade-offs (see above). That several of the species with high drought resistance were associated with moist habitats additionally implies that they were not systematically excluded from moist habitats due to other possible trade-offs with drought stress tolerance, e.g. against competitive ability, herbivore or pathogen defenses (Grime et al. 1997, Haugen et al. 2008). On the other hand, our data also do not support the 'physiological tolerance' hypothesis, which postulates that resistant species that are able to withstand extreme conditions are widely distributed (Currie et al. 2004). Other factors that differ between habitats with different water availability must limit the distribution of the drought resistant species to habitats with various different moisture conditions. For example, high nitrogen requirements may limit the distribution of a drought resistant species to water regimes that are suitable to high microbial nitrogen mineralization rates and at the same time allow high nitrogen uptake with the transpiration stream (Araya et al. 2013). Complex interactions of drought resistance with other factors, such as nutrients, can accelerate or dampen effects of water availability (e.g. Eskelinen and Harrison 2015), and may limit species to specific parts of the gradient. Additionally, plant-plant interactions, i.e. competition and facilitation, are known to play an important role in performance of grassland species across soil water availability gradients and to influence species distributions (e.g. Brooker et al. 2008). Thus, multiple assembly processes, i.e. environmental resistance (environmental filtering) and plant-plant interactions, could operate simultaneously to structure plant communities along a resource gradient (Spasojevic and Suding 2012).

Our results on temperate grassland plants on one hand differed from findings from moist tropical forests where species whole-plant drought resistance had a pronounced direct effect on their distribution along rainfall gradients (Engelbrecht et al. 2007, Esquivel-Muelbert et al. 2017a), underlining that the relative importance of mechanisms for species distributions differs across ecosystems. On the other hand, consistent with our results, drought resistant species were also not excluded from even wet tropical forests, entailing that potential trade-offs of drought resistance with other factors were also weak and did not limit species distribution (Esquivel-Muelbert et al. 2017b).

To further our understanding of the importance of individual species drought responses for their distribution as well as for community composition and ecosystem functions across moisture gradients in grasslands, we need to go beyond the coarse grained non-quantitative classification of habitat association and link species drought resistance to abundance changes in response to temporal and spatial variation of soil water potentials. We will also have to explicitly consider interactions with species responses to additional abiotic and biotic factors on one hand, and environmental co-variation of these factors on the other hand. Linking drought resistance of individual species, i.e. their fundamental niche, with their responses to drought in community level experiments focusing on neighbor or diversity effects (e.g. Isbell et al. 2015, Herz et al. 2017) as well as in natural communities (e.g. Tilman and El Haddi 1992, Bütof et al. 2012), will allow to further elucidate the traits and factors shaping species distributions across moisture gradients, i.e. their realized hydrological niche.

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Conflict of interest – There are no conflicts of interest to declare.

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Supporting information

Table S1 List of the 44 study species. Given are the species' code, family, life form (grasses and forbs, including legumes, L), the Ellenberg indicator value for moisture (M-value, Ellenberg et al. 1992, 'X' indicates 'indifferent'), sample sizes at the beginning of the experiment, number of survivors (in parenthesis is the number of individuals where all above-ground biomass was dead after drought but that resprouted in the following year) and sample sizes of relative growth rate (in parenthesis is number of individuals where all above-ground biomass died during the dry treatment). In species where the initial sample number for survival was below 36, the full number of seedlings was not available for transplanting, for species with initial sample numbers above 36 a transplanting error had occurred. The last three species (CAPSBU, MEDILU, TRIFRE) were excluded throughout all analyses presented in the main text because of their poor performance in the irrigated plots.

Species	Code	Family	Life form	M	Survival				Growth	
					# Initial		# Survivors		n	
					Wet	Dry	Wet	Dry	Wet	Dry
Achillea millefolium L.	ACHIMI	Asteraceae	Forb	4	37	34	37	34	6	5
Agrostis capillaris L.	AGROCA	Poaceae	Grass	x	37	37	37	30 (6)	6	6 (2)
Agrostis stolonifera L.	AGROST	Poaceae	Grass	7	35	36	35	35 (1)	5	6
Alopecurus pratensis L.	ALOPPR	Poaceae	Grass	6	33	35	33	32 (6)	6	6 (1)
Anthoxanthum odoratum L.	ANTHOD	Poaceae	Grass	x	36	37	36	36 (1)	6	6 (1)
Arrhenatherum elatius (L.) J. & K.	ARRHEL	Poaceae	Grass	x	36	34	36	32 (9)	6	6 (1)
Brachypodium pinnatum (L.) PB.	BRACPI	Poaceae	Grass	4	35	36	32 (1)	30 (2)	6	6
Briza media L.	BRIZME	Poaceae	Grass	x	36	35	36	31 (6)	6	6
Bromus hordeaceus L. s. str.	BROMHO	Poaceae	Grass	x	37	35	37	32 (3)	6	6 (1)
Centaurea jacea L.	CENTJA	Asteraceae	Forb	x	32	36	32	34	6	6
Cerastium holosteoides Fr.	CERAHO	Caryophyllaceae	Forb	5	37	36	37	24 (8)	6	6 (2)
Cirsium oleraceum (L.) Scop.	CIRSOL	Asteraceae	Forb	7	13	26	13	25	3	3
Crepis biennis L.	CREPBI	Asteraceae	Forb	6	36	36	36	35 (1)	6	6
Dactylis glomerata L. ssp. *glomerata*	DACTGL	Poaceae	Grass	5	36	36	35 (1)	35 (1)	6	6
Daucus carota L. ssp. *Carota*	DAUCCA	Apiaceae	Forb	4	39	35	39	35 (1)	6	6

Elymus repens (L.) Gould	ELYMRE	Poaceae	Grass	x	35	36	35	34 (6)	6	6 (2)
Festuca guestfalica Boenn. Ex Rchb.	FESTGU	Poaceae	Grass	4	35	35	35	33 (1)	5	6
Festuca ovina L. agg.	FESTOV	Poaceae	Grass	x	37	35	37	35	6	6
Festuca pratensis Huds. ssp. *pratensis*	FESTPR	Poaceae	Grass	6	35	35	34	33	6	6 (1)
Festuca rubra L.	FESTRU	Poaceae	Grass	6	36	35	36	35 (2)	6	6
Galium mollugo L. agg.	GALIMO	Rubiaceae	Forb	4	34	35	34	35 (1)	6	6
Helictotrichon pubescens (Huds.) Pilg. ssp. *pubescens*	HELIPU	Poaceae	Grass	3	36	34	36	34 (1)	6	6 (1)
Holcus lanatus L.	HOLCLA	Poaceae	Grass	6	37	38	37	37	6	6
Lathyrus pratensis L.	LATHPR	Fabaceae	Forb (L)	6	34	31	34 (2)	24 (4)	4	5
Leontodon hispidus L.	LEONHI	Asteraceae	Forb	5	33	35	33 (2)	34	5	6
Leucanthemum vulgare Lam.	LEUCVU	Asteraceae	Forb	4	36	37	36	37	6	6
Lolium perenne L.	LOLIPE	Poaceae	Grass	5	36	36	36	36 (1)	6	6
Lotus corniculatus L.	LOTUCO	Fabaceae	Forb (L)	4	36	35	36	35 (1)	6	6
Phleum pratense L.	PHLEPR	Poaceae	Grass	5	36	35	36 (1)	28 (3)	6	6 (2)
Plantago lanceolate L.	PLANLA	Plantagina -ceae	Forb	x	36	36	36	36	6	6
Poa pratensis L.	POAPR	Poaceae	Grass	5	35	33	35	26 (3)	6	5
Poa trivialis L.ssp. *trivialis*	POATR	Poaceae	Grass	7	37	31	34	13 (7)	6	6 (4)
Prunella vulgaris L.	PRUNVU	Lamiaceae	Forb	5	35	31	34	29 (4)	6	6 (1)
Ranunculus acris L.	RANUAC	Ranuncula-ceae	Forb	6	29	30	28	23 (3)	6	5
Ranunculus bulbosus L. ssp. *bulbosus*	RANUBU	Ranuncula-ceae	Forb	3	12	13	11	10	3	3
Ranunculus repens L.	RANURE	Ranuncula-ceae	Forb	7	36	36	35	36 (6)	6	6
Rumex acetosa L.	RUMEAC	Polygonaceae	Forb	x	37	36	36	36 (1)	6	6 (1)
Rumex crispus L.	RUMECR	Polygonaceae	Forb	7	35	36	35	35	6	6
Taraxacum sect. *Ruderalia* Kirschner H.Øllg. &Štěpánek	TARARU	Asteraceae	Forb	5	36	36	36	36 (4)	6	5
Trisetum flavescens (L.) PB.	TRISFL	Poaceae	Grass	x	34	35	33	25 (10)	6	6 (1)
Vicia cracca L.	VICICR	Fabaceae	Forb (L)	6	37	35	37 (2)	25 (3)	6 (1)	6 (4)

Capsella bursa-pastoris (L.) Medik.	CAPSBU	Brassicaceae	Forb	5	27	26	10	4		5 (1)	3 (1)	
Medicago lupulina L.	MEDILU	Fabaceae	Forb (L)	4	35	30	24	16	4		6 (1)	
Trifolium repens L.	TRIFRE	Fabaceae	Forb (L)	5	33	32	22	4 (2)	5 (1)		6 (5)	

Table S2 Comparison of monthly rainfall and average temperature during the experimental period in 2015 with long-term averages (1881−2010, data EBG).

	Rainfall (mm)		Temperature (°C)	
	2015	long-term average	2015	long-term average
July	32	92	19.9	17.6
August	40	64	20.5	16.6
September	25	64	12.7	12.6

Table S3 Treatment and species effects on plant performances for all 44 species (including the ones with poor performance under irrigated conditions excluded in the main analyses) and for growth based on surviving individuals only (RGR_s) or on all individuals (RGR_{all}), including the dead ones (living leaf area = zero). Generalized linear mixed models (GLMM, for survival) and linear mixed models (LMM, for growth) were used with treatment, species and treatment x species as fixed factors and block and plot as random effect. Models were set up over all species and for the two life forms (grasses and forbs) separately. The three species excluded were all forbs and therefore the results from grasses presented here are same as those presented in Table 2. For fixed effects Chi^2 (X^2) and F are given for survival and RGR, respectively, and for random effect X^2 are given. Significance levels are presented as (*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Note that all results for RGR are qualitatively the same as in Table 2, but treatment effect became significant for survival of forbs when the three species were included.

	Treatment	Species	Treatment x species	Block and plot
Survival (44 species)				
All	55.16**	309.44***	34.47	83.81***
Grasses	17.09*	71.35***	11.6	33.37***
Forbs	37.11***	184.1***	19.61	16.75***
RGR_s (44 species)				
All	6.89(*)	2.47***	0.94	26.26***
Grasses	12.05*	1.11	1.19	6.07*
Forbs	3.57	3.35***	0.78	14.34***
RGR_{all} (44 species)				
All	9.85*	2.68***	1.24	25.85***
Grasses	13.64*	1.29	1.43	9.35**
Forbs	4.51	3.8***	1.19	22.46***
RGR_{all} (41 species)				
All	10.77*	3***	1.41(*)	22.9***
Grasses	13.64*	1.29	1.43	9.35**
Forbss	5.05(*)	4.81***	1.46	17.86***

Table S4 Drought effects on individual species (compare Fig. 2A, 3A and S2A). Generalized mixed models (for survival) and linear mixed model (for growth) with treatment as fixed effect and block as random effect were set up for each species. Values for both fixed and random effect values are Chi^2 (X^2). Significant effects are marked bold, and significance levels are given as (*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Where all individuals survived in both treatments, no values are given. RGR was analyzed in all species for survivors only (RGR_s) and for all individuals, including the dead ones (RGR_{all}). For species codes, see Table S1. Grasses and forbs are indicated with G and F, respectively.

Species code	**Survival**		**RGR_s**		**RGR_{all}**	
	Treatment	**Block**	**Treatment**	**Block**	**Treatment**	**Block**
ACHIMI (F)	-	-	1.89	0	-	-
AGROCA (G)	**4.03***	0.24	1.75	0.06	**4.17***	0.03
AGROST (G)	0.31	0	0.001	0	-	-
ALOPPR (G)	1.53	0	2.09	0	**3.51(*)**	0
ANTHOD (G)	0.31	0	0.22	0	0. 53	0
ARRHEL (G)	0.49	0.93	1.12	0	2.07	0
BRACPI (G)	0.4	0	1.04	0	-	-
BRIZME (G)	2.46	0	0.18	0	-	-
BROMHO (G)	1.8	0	**8.52****	0	**8.61****	0.34
CENTJA (F)	0.77	0	0.08	0	-	-
CERAHO (F)	**6.41****	0.53	**9.81****	**3.27(*)**	**17.92*****	0
CIRSOL (F)	0.10	0.20	0.03	0.16	-	-
CREPBI (F)	0.33	0	1.3	0	-	-
DACTGL (G)	0	0	**6.13***	0	-	-
DAUCCA (F)	-	-	0.59	0	-	-
ELYMRE (G)	0.91	0	**5.33***	2.21	**8.29****	2.07
FESTGU (G)	0.96	0	**2.72(*)**	0	-	-
FESTOV (G)	-	-	0.45	0	-	-
FESTPR (G)	0.34	0	0.28	0.13	0.81	0.31
FESTRU (G)	-	-	1.27	2.17	-	-
GALIMO (F)	-	-	0.45	0	-	-
HELIPU (G)	-	-	**6.06***	0	**7.81****	0
HOLCLA (F)	0.31	0	1.33	0	-	-

LATHPR (F)	**4.34***	0	0.99	0.01	-	-
LEONHI (F)	0.002	0	0.09	0.13	-	-
LEUCVU (F)	-	-	0.39	0	-	-
LOLIPE (G)	-	-	**15.86*****	0	-	-
LOTUCO (F)	-	-	**5.33***	0	-	-
PHLEPR (G)	**3.89***	0	**4.67***	0	**8.15****	0.32
PLANLA (F)	-	-	3.00(*)	0	-	-
POAPR (G)	3.28(*)	0.48	**4.75***	0	-	-
POATR (G)	**9.26****	1.48	2.74(*)	0	**13.21*****	1.85
PRUNVU (F)	0.46	0	**4.32***	3.75(*)	**4.92***	2.49
RANUAC (F)	3.76(*)	0	**4.26***	0	-	-
RANUBU (F)	0.16	0	1.59	0	-	-
RANURE (F)	0.0019	0	**11.5*****	0.61	-	-
RUMEAC (F)	0.0024	0	2.03	0.80	2.89(*)	0.77
RUMECR (F)	0.4×10^{-4}	0	0.05	0.08	-	-
TARARU (F)	-	-	1.86	0.29	-	-
TRISFL (G)	3.58(*)	**6.50****	**20.81*****	0	**26.04*****	0
VICICR (F)	**6.09****	0	**4.47***	0	-	-
CAPSBU (F)	1.06	2.95(*)	1.04	0	0.51	0
MEDILU (F)	1.57	0	0.70	0	1.20	0
TRIFRE (F)	**19.54*****	0	0.001	**4.13***	2.02	1.35

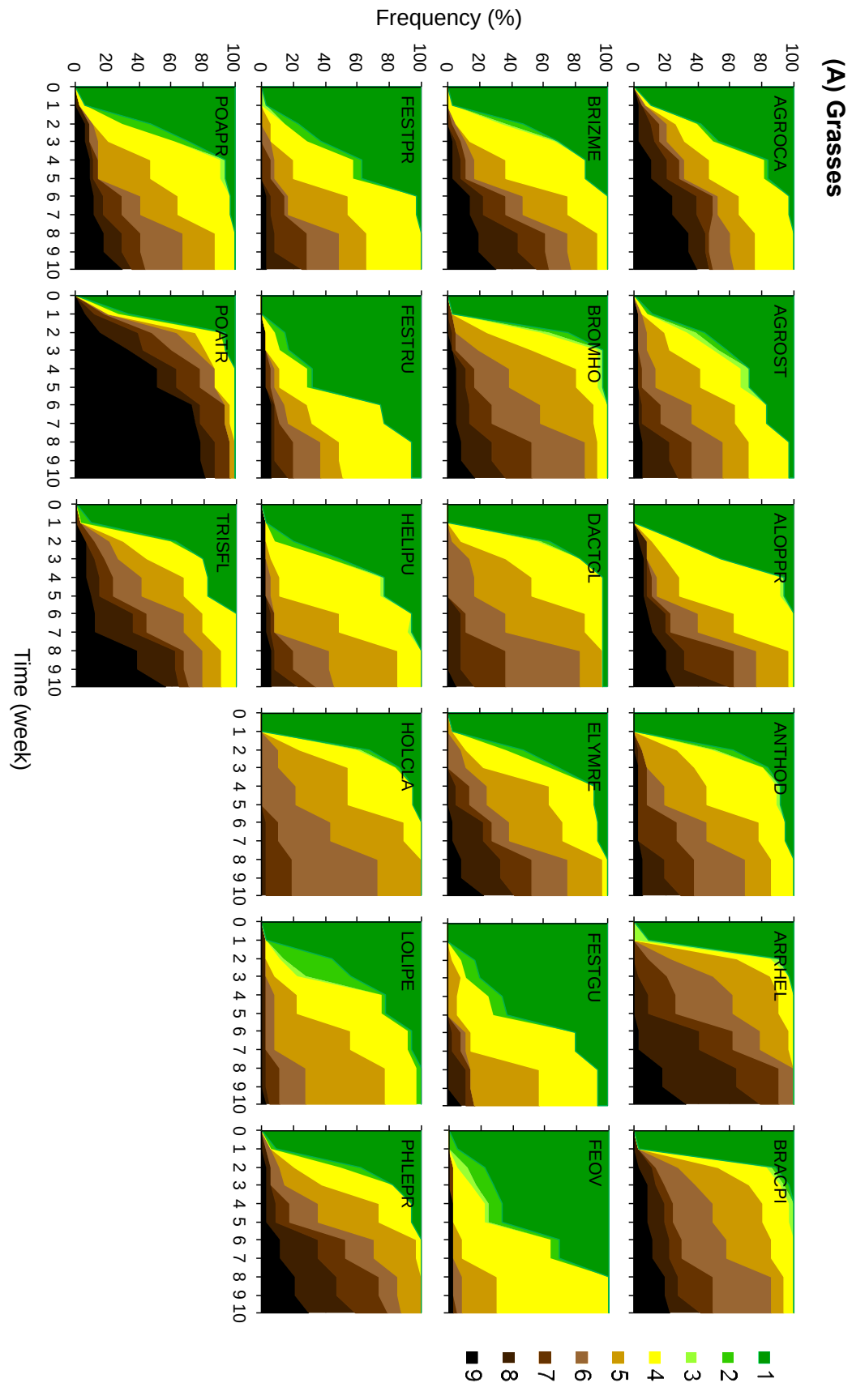
Table S5 Compilation of correlations and regressions of performance parameters over 41 species (compare Fig. 4 and 5), and over all 44 species including the three species excluded from the main analyses, i.e. CAPSBU, MEDILU, TRIFRE. Relative growth rates were calculated only for surviving individuals (RGR_s) and for all individuals including dead ones (RGR_{all}). All analyses were conducted separately for grasses and forbs, and combining both life forms. Given are Pearson correlation coefficients (r) between drought resistance of RGR_s ($DR_{growth.surv}$) and RGR_{all} ($RR_{growth.all}$) and regression coefficients (R^2) for the rest of the performance parameters, the p-values and sample sizes. n.s. indicates not significant at $p > 0.1$. Species without specific moisture associations, i.e. Ellenberg indicator values (EIV) for soil moisture (M-value) = X (see Table 1), were excluded for the regressions of habitat association on drought sensitivity. The results for 41 species and 44 species are same for grasses since only forb species were excluded from the main analyses. Note that all correlations and regressions are qualitatively same between the analyses of 41 vs. 44 species, and the analyses of RGR_{all} vs. RGR_s except that the relation between DR_{growth} and EIV for grasses was significant only for surviving individuals, not for all individuals including dead ones.

		41 species			**44 species**		
Correlation		**r**	**p**	**n**	**r**	**p**	**n**
$Log(DR_{growth.surv})$ vs. $Log(DR_{growth.all})$	All	0.96	< 0.001	41	0.84	< 0.001	44
	Grasses	0.82	< 0.001	21	-	-	-
	Forbs	0.98	< 0.001	20	0.85	< 0.001	23
Regression		**R^2**	**p**	**n**	**R^2**	**p**	**n**
$DR_{survival}$ vs. $log(DR_{growth.surv})$	All	0.10	0.059	41	0.12	0.024	44
	Grasses	0.09	n.s.	21	-	-	-
	Forbs	0.14	0.098	20	0.20	0.031	23
$RGR_{wet.surv}$ vs. $DR_{survival}$	All	0.01	n.s.	41	0.03	n.s.	44
	Grasses	0.03	n.s.	21	-	-	-
	Forbs	0.14	0.099	20	0.11	n.s.	23
$RGR_{wet.all}$ vs. $DR_{survival}$	All	0.01	n.s.	41	0.04	n.s.	44
	Grasses	0.03	n.s.	21	-	-	-
	Forbs	0.14	0.100	20	0.12	0.091	23
$RGR_{wet.surv}$ vs. $RGR_{dry.surv}$	All	0.07	0.085	41	0.03	n.s.	44
	Grasses	0.05	n.s.	21	-	-	-
	Forbs	0.32	0.007	20	0.15	0.062	23

$RGR_{wet.all}$ vs. $RGR_{dry.all}$	All	0.07	0.090	41	0.11	0.026	44
	Grasses	0.01	n.s.	21	-	-	-
	Forbs	0.29	0.011	20	0.34	0.003	23
$DR_{survival}$ vs. $\log(DR_{growth.all})$	All	0.19	0.005	41	0.01	n.s.	44
	Grasses	0.36	0.005	21	-	-	-
	Forbs	0.19	0.050	20	0.03	n.s.	23
$DR_{survival}$ vs. EIV	All	0.05	n.s.	30	0.01	n.s.	33
	Grasses	0.12	n.s.	13	-	-	-
	Forbs	0.01	n.s.	17	0.00	n.s.	20
$Log(DR_{growth.surv})$ vs. EIV	All	0.04	n.s.	30	0.02	n.s.	33
	Grasses	0.15	n.s.	13	-	-	-
	Forbs	0.13	n.s.	17	0.05	n.s.	20
$Log(DR_{growth.all})$ vs. EIV	All	0.07	n.s.	30	0.01	n.s.	33
	Grasses	0.00	n.s.	13	-	-	-
	Forbs	0.12	n.s.	17	0.01	n.s.	20
S_{dry} vs. EIV	All	0.03	n.s.	30	0.00	n.s.	33
	Grasses	0.10	n.s.	13	-	-	-
	Forbs	0.00	n.s.	17	0.01	n.s.	20

Table S6 Relations of the % individuals with severe visual drought damage (drought-damage category 5−9, see Table 2) at various times into the drought with % survival of 10 weeks of drought (S_{dry}) and drought resistance of survival ($DR_{survival}$) and growth for surviving individuals ($DR_{growth.surv}$) and for all individuals including dead ones ($DR_{growth.all}$). % individuals were log transformed. Shown are R^2 values and significance level as (*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, over 41 species. Significant relations after Bonferroni correction are in bold. All results were qualitatively same when considering all 44 species (data not shown).

Week	**All**	**Grasses**	**Forbes**	**All**	**Grasses**	**Forbes**
	Log(% damaged individuals) vs. S_{dry}			Log(% damaged individuals) vs. Log($DR_{growth.surv}$)		
2	**0.36*** **	**0.45*** **	0.27*	0.00	0.02	0.00
3	**0.44*** **	**0.50*** **	**0.45** **	0.07(*)	0.06	0.11
4	**0.43*** **	**0.42** **	**0.54*** **	0.04	0.01	0.07
5	**0.36*** **	**0.33** **	**0.46*** **	0.11*	0.02	0.20*
6	**0.36*** **	**0.33** **	**0.44*** **	0.13*	0.02	0.23*
7	**0.31*** **	**0.36*** **	**0.36*** **	**0.28*** **	0.01	0.04
8	**0.28*** **	**0.36** **	0.28*	0.03	0.01	0.05
9	0.16**	0.18*	0.20*	0.03	0.01	0.04
	Log(% damaged individuals) vs. $DR_{survival}$			Log(% damaged individuals) vs. Log($DR_{growth.all}$)		
2	**0.36*** **	**0.42** **	0.30**	0.02	0.11	0.01
3	**0.45*** **	**0.50*** **	**0.41** **	0.10*	0.14(*)	0.11
4	**0.42*** **	**0.39** **	**0.47*** **	0.08(*)	0.12	0.09
5	**0.35*** **	0.32**	**0.38** **	0.15*	0.11	0.20*
6	**0.34*** **	0.32**	**0.37** **	0.17**	0.11	0.23*
7	**0.28*** **	**0.37** **	0.25*	0.05	0.06	0.05
8	**0.25*** **	**0.37** **	0.20*	0.05	0.06	0.05
9	0.14*	0.18(*)	0.13	0.04	0.05	0.04


(A) Grasses
Frequency (%)
Time (week)
AGROCA
AGROST
ALOPPR
ANTHOD
ARRHEL
BRACPI
BRIZME
BROMHO
DACTGL
ELYMRE
FESTGU
FEOV
FESTPR
FESTRU
HELIPU
HOLCLA
LOLIPE
PHLEPR
POAPR
POATR
TRISFL
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2
3
4
5
6
7
8
9

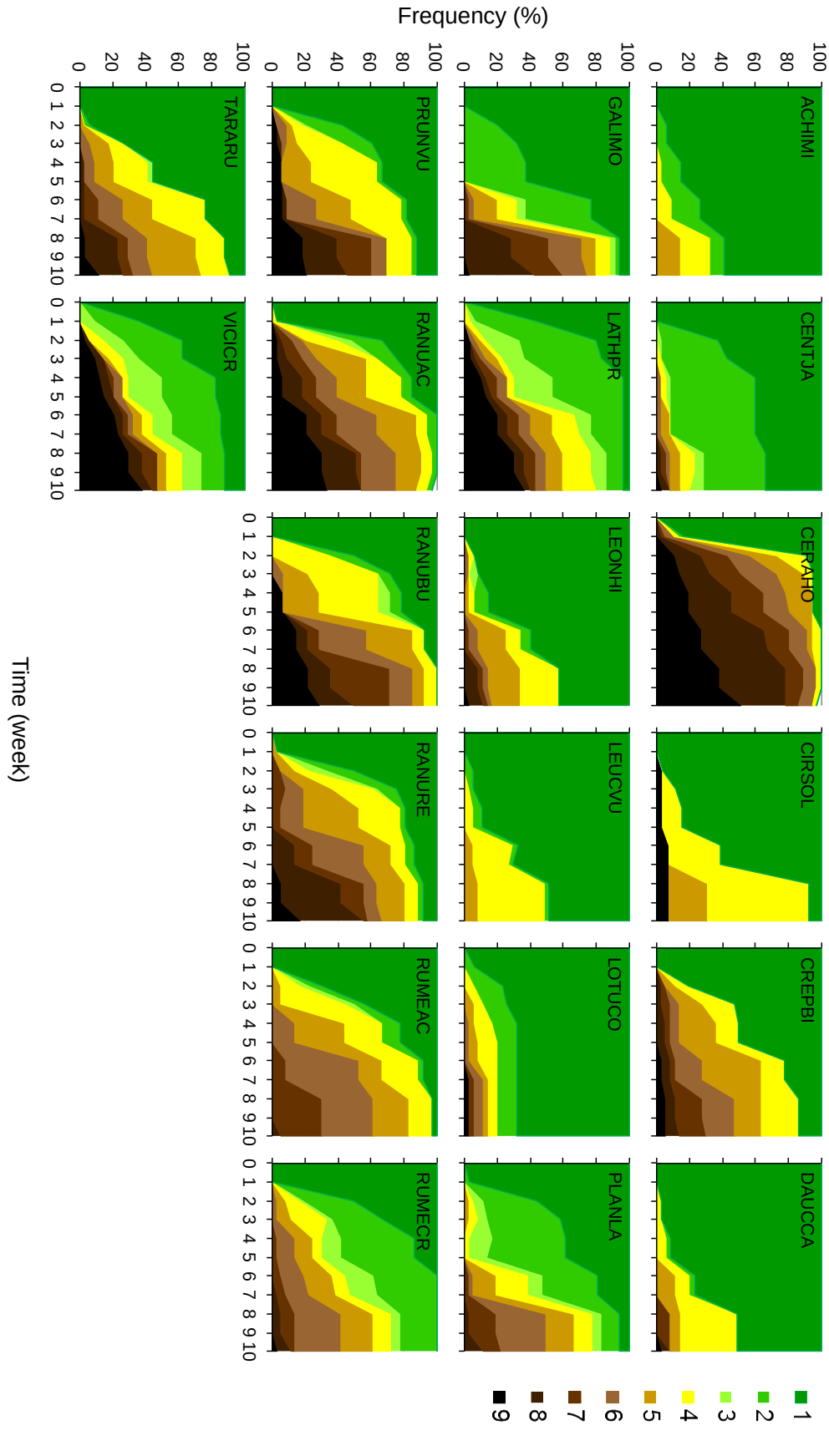
(B) Forbs
Frequency (%)
Time (week)
ACHIMI
CENTJA
CERAHO
CIRSOL
CREPBI
DAUCCA
GALIMO
LATHPR
LEONHI
LEUCVU
LOTUCO
PLANLA
PRUNVU
RANUAC
RANUBU
RANURE
RUMEAC
RUMECR
TARARU
VICICR
1
2
3
4
5
6
7
8
9

Figure S1 Progression of visual drought damage from wilting through leaf tip drying to above-ground mortality in 41 grassland species. Species are sorted alphabetically, separately for grasses (A) and forbs (B). For species codes, see Table S1. For a description of the drought damage stages, see Table 2.

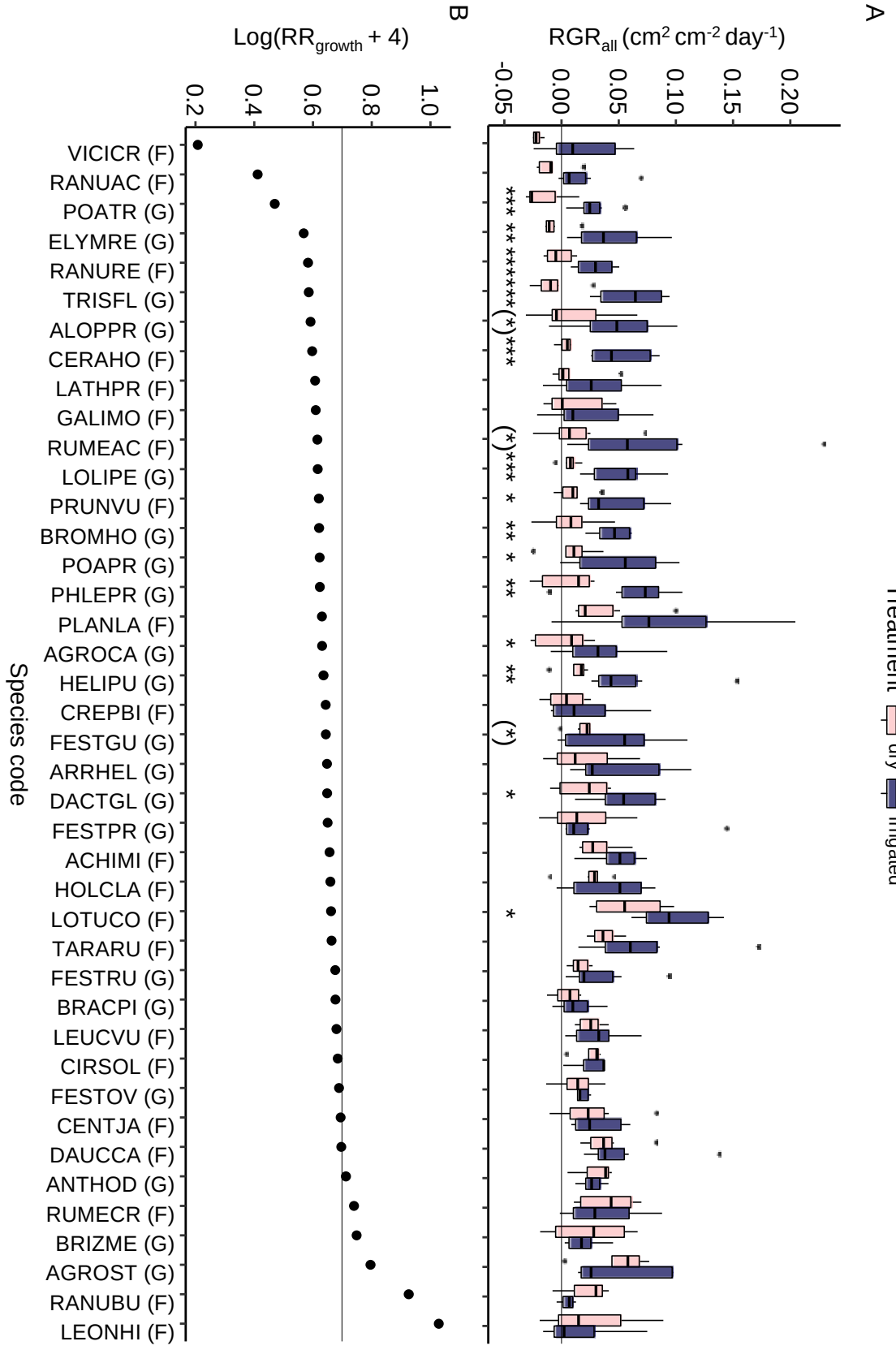
A
B
RGR$_{all}$ (cm^2 cm^{-2} day^{-1})
Log(RR$_{growth}$ + 4)
-0.05
0.00
0.05
0.10
0.15
0.20
0.2
0.4
0.6
0.8
1.0
Species code
VICICR (F)
RANUAC (F)
POATR (G)
ELYMRE (G)
RANURE (F)
TRISFL (G)
ALOPPR (G)
CERAHO (F)
LATHPR (F)
GALIMO (F)
RUMEAC (F)
LOLIPE (G)
PRUNVU (F)
BROMHO (G)
POAPR (G)
PHLEPR (G)
PLANLA (F)
AGROCA (G)
HELIPU (G)
CREPBI (F)
FESTGU (G)
ARRHEL (G)
DACTGL (G)
FESTPR (G)
ACHIMI (F)
HOLCLA (F)
LOTUCO (F)
TARARU (F)
FESTRU (G)
BRACPI (G)
LEUCVU (F)
CIRSOL (F)
FESTOV (G)
CENTJA (F)
DAUCCA (F)
ANTHOD (G)
RUMECR (F)
BRIZME (G)
AGROST (G)
RANUBU (F)
LEONHI (F)
Treatment
dry
irrigated

Figure S2 (A) Relative growth rate (RGR, $cm^2cm^{-2}day^{-1}$) for 41 fobs and grass species under dry and irrigated conditions (red and blue boxes, respectively) including both surviving and dead individuals (RGR_{all}). RGR was based on projected living leaf area. Results of treatment effects on growth within each species are given below the box plots ((*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), for details see Tables S3 and S4. **(B)** Drought resistance of growth (DR_{growth}) based on the response ratio between dry and irrigated conditions for all individuals (alive and dead). Values below the line at 0.7 indicate a decrease in RGR in the dry relative to the irrigated treatment. Species are sorted by increasing drought resistance. Grasses and forbs are indicated as G and F, respectively. For species codes and sample size, see Table S1.

Manuscript 2: High turgor loss point promotes drought survival of temperate grassland species

High turgor loss point promotes drought survival of temperate grassland species

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Abstract

Understanding the mechanisms underlying differential drought resistance of temperate grassland species is crucial to predict consequences of global climate change. Turgor loss point (π_{tlp}) has been suggested to be a key drought resistance trait in woody species. In herbaceous species its role has not yet been tested. We first validated the osmometer method to assess π_{tlp} in herbaceous species. We then assessed π_{tlp} of 41 temperate grassland species common in Germany (20 forbs, 21 grasses), and directly related them to species' whole-plant survival and midday leaf water potentials under drought in a common garden experiment. Species drought survival increased with increasing π_{tlp} in forbs and grasses. Moreover, midday leaf water potential under drought was positively related to π_{tlp} and drought survival. Our results indicate that π_{tlp} is a key trait that promotes drought resistance of grassland species by enabling the maintenance of high water potentials under drought, i.e. a desiccation avoidance strategy. The positive relations of π_{tlp} to drought resistance in forbs and grasses were opposite to the negative relation found in woody plants, indicating that mechanisms of drought resistance differ fundamentally between woody and herbaceous species.

Keyword: drought resistance, drought tolerance, forbs, functional traits, grasses, midday leaf water potential, water relations.

Introduction

Grasslands occupy one-fifth of the world's land surface, store about a third of terrestrial carbon, and provide important goods and services, such as food, forage, livestock and biodiversity (Scurlock & Hall, 1998; Gibson, 2009; Hoover & Rogers, 2016). Grasslands worldwide are influenced by drought (Gibson, 2009), which alters species abundances across time and space, shapes species distribution patterns and community composition, and reduces productivity (Weaver, 1968; Tilman & El Haddi, 1992; Buckland *et al.*, 1997). Under climate change, precipitation patterns are projected to change, and the frequency and severity of droughts are expected to increase (IPCC, 2014), enhancing effects of drought on grasslands.

A large number of experimental studies investigating effects of drought on grasslands exist, which mostly focus on ecosystem properties and how they are affected by community composition. They consistently show that the response and resilience of ecosystems to drought are influenced by community trait and species composition, and by diversity (e.g. Tilman & Downing, 1994; Bai *et al.*, 2004; Wu *et al.*, 2011; Isbell *et al.*, 2015; Wagg *et al.*, 2017). However, much less attention has been dedicated to understanding how in turn drought affects species performance and the composition and diversity of grasslands (Tilman & El Haddi, 1992). Drought resistance - the capacity to survive periods of low water availability - varies widely among species within and across plant communities, including in grasslands (e.g. Buckland *et al.*, 1997). To predict the response of grassland communities to changing drought regimes, a thorough understanding of the differential drought resistance of grassland species and the underlying mechanisms is necessary.

Plants exhibit a wide range of morphological, anatomical and physiological mechanisms that allow them to withstand drought. Mechanisms of whole-plant drought resistance can be categorized into (1) mechanisms of desiccation tolerance, which allow plants to sustain physiological activities despite of low water potentials, and (2) mechanisms of desiccation avoidance, which allow plants to maintain high water potentials during drought through maximizing water uptake and water storage, and minimizing water loss (Levitt, 1972; Vilagrosa *et al.*, 2012; Comita & Engelbrecht, 2014). Efficient avoidance of desiccation leads to the maintenance of high midday leaf water potentials under drought (Ψ_{MD}), which thus provides a comparative measure of desiccation avoidance across species (Comita & Engelbrecht, 2014).

The question which mechanisms and their combinations are important for species differential drought resistance has been a focus of a large body of research (e.g. Chaves *et al.*, 2003; McDowell *et al.*, 2008, Skelton *et al.*, 2015). Various morphological traits, e.g. leaf area, specific leaf area, leaf dry matter content and seed mass, as well as leaf water potential at stomatal closure are broadly related to habitat affinities with respect to soil moisture in forbs and grasses (Tucker *et al.*, 2011; Craine *et al.*, 2013; Shipley *et al.*, 2017; Belluau & Shipley, 2018). However, relations remain broad and the insight into the functional role of the traits limited. Recent studies have tested linkages between additional physiological and morphological functional traits and various drought responses, e.g. leaf mortality, or/and habitat water availability (Zwicke *et al.*, 2015; Ocheltree *et al.*, 2016; Farrell *et al.*, 2017; Belluau & Shipley, 2017). However, rigorous tests of the role of different mechanisms for drought resistance require to explicitly directly relate traits and their combinations to comparative assessment of whole-plant survival under drought. Surprisingly, such studies remain exceedingly scarce and restricted to woody species (e.g. Engelbrecht *et al.*, 2007; Kursar *et al.*, 2009; Brenes-Arguedas *et al.*, 2013, Urli *et al.*, 2013, O'Brien *et al.*, 2017). Many grassland species can resprout after drought from surviving belowground organs even after complete aboveground mortality, and leaf abscission may even promote drought performance by minimizing water loss (Volaire *et al.*, 1998). Direct linkages to whole-plant drought survival are therefore especially important in grassland species for evaluating the mechanisms of drought resistance and impacts of drought. To our knowledge, to date no study has directly related potentially important mechanisms of drought resistance to whole-plant drought survival in temperate grassland species, severely limiting our ability to predict consequences of drought for these important systems.

Turgor loss point (π_{tlp}), the leaf water potential at which the turgor pressure of leaf cells equals zero, has long been considered a crucial parameter in plant water relations (Cheung *et al.*, 1975). It varies widely among plant species and has more recently been suggested to be a useful proxy of species drought resistance (Bartlett *et al.*, 2012b). A *low* (more negative) turgor loss point can allow the leaf to remain turgid despite of decreasing leaf water potential (Ψ_{leaf}), and, therefore, maintain photosynthesis, water transport, transpiration and growth, conferring high drought resistance as a mechanism of desiccation tolerance (Bartlett *et al.*, 2012b). On the other hand, a *high* (less negative) turgor loss point may also promote drought resistance by leading to early stomatal closure and thus enabling plants to maintain high water potential and

hydration even under decreasing soil water status (Bartlett *et al.*, 2012b), therefore reflecting mechanisms of desiccation avoidance.

A *low* turgor loss point has been shown to be associated with increasing dryness across biomes (Bartlett *et al.*, 2012b), with increasing regional and local occurrence under dry conditions along rainfall, topographical and soil texture gradients (Lenz *et al.*, 2006; Mitchell *et al.*, 2008; Maréchaux *et al.*, 2015), and with a high survival ratio under natural drought across subtropical tree species (Zhu *et al.*, 2017). Together, these studies provide strong evidence that a low turgor loss point can indeed promote drought resistance as a mechanism of desiccation tolerance.

Studies of turgor loss point as an indicator of drought resistance have almost exclusively focused on woody plants, e.g. recent comprehensive meta-analyses on turgor loss point and its relation to drought regimes or further drought related traits included 93 and 100% woody species, respectively (Bartlett *et al.*, 2012b, Bartlett *et al.*, 2016). In contrast, the knowledge about the association of turgor loss point with drought resistance in herbaceous species, and specifically in grassland species, is limited and inconsistent. A study of 33 C4 grasses found the opposite trend than the one in woody species, with species that occupy drier habitats exhibiting higher turgor loss points than those in wetter habitats (Liu & Osborne, 2014). In contrast, across 8 C4 grasses the water potential at which 50% of the leaves died was marginally positively correlated with the turgor loss point (Ocheltree *et al.*, 2016), and across 12 pea cultivars (*Pisum sativum* L.) the growth of epicotyls under conditions of low water potentials was higher in cultivars with low turgor loss point (Sánchez *et al.*, 2004) – both results consistent with the patterns in woody species. In further studies, turgor loss point did not show any relation with dryness across habitats (9 C4 grasses, Ocheltree *et al.*, 2016 and 7 herbaceous species, Farrell *et al.*, 2017) or biomes (18 herbaceous species, reanalyzed from Bartlett *et al.*, 2012b). In summary, the role of the turgor loss point for drought responses in herbaceous species remains inclusive, and its potential as a proxy of drought resistance is unclear.

The use of turgor loss point to infer plant drought resistance became especially appealing by the development of a method for its rapid assessment. Bartlett *et al.* (2012a) showed that in woody species turgor loss point can be estimated from measurements of the osmotic potential at full turgor with an osmometer. This 'osmometer method' makes turgor loss point measurement 30 times faster than traditional approaches using pressure-volume curves (Tyree & Hammel, 1972; Bartlett *et al.*, 2012a). However, whether turgor loss point can be reliably

assessed by osmometer measurements of the osmotic water potential at full turgor in herbaceous species has not yet been verified.

In our study, we first validated the 'osmometer method' for forbs and grasses by relating turgor loss point measured with traditional pressure-volume curves (Tyree & Hammel, 1972) to osmotic water potential at full turgor assessed with an osmometer (Bartlett *et al.*, 2012a). We then tested two alternative hypotheses for the linkage of turgor loss point with drought resistance in grassland species: (1) a *low* turgor loss point is associated with high species drought survival and unrelated to midday leaf water potential, implying a strategy of desiccation tolerance consistent with woody species; or (2) a *high* turgor loss point is associated with high species drought survival and a high midday leaf water potential, indicating the importance of desiccation avoidance mechanisms, a strategy contrasting to woody species.

To test these hypotheses, we assessed the turgor loss points for 41 temperate grassland species and related them to their midday leaf water potentials and to their whole-plant survival in a field common garden drought experiment.

Materials and Methods

Study species

We initially chose 43 temperate grassland species (Table S1), 22 forbs and 21 grasses, common in Germany. Species were selected based on the following criteria: (1) high abundance and frequency in 150 long-term grassland plots (4m × 4m) in Biodiversity Exploratories from three areas across Germany (Schorfheide-Chorin, Hainich-Dün and Schwäbische Alb; Fischer *et al.*, 2010), (2) to include different life forms i.e. forbs and grasses, (3) to represent a wide range of moisture associations based on Ellenberg's soil moisture index (F-value from 3 to 7, Ellenberg *et al.*, 1991), and (4) to be perennial, the dominant life history strategy in European temperate grasslands (Ellenberg *et al.*, 1991). The selected species were from 34 genera and 9 families and all have C3 photosynthesis (Collatz *et al.*, 1998). Seeds were bought from a commercial supplier (Rieger-Hofmann GmbH and Saaten Zeller, Germany) and germinated in a greenhouse in mid-March, 2015. Plants were transplanted to larger pots for the measurement of turgor loss point in the greenhouse and to a field common garden drought experiment for the assessments of comparative midday leaf water potential and whole-plant drought survival in mid-June 2015.

Two of the original 43 species established poorly before transplanting to the field and showed poor performance even in well-watered plots (< 70% survival, *Medicago lupulina L.* and *Trifolium repens L.*). We therefore excluded these species from the analyses presented in the main text. However, analyzing the data including or excluding these species yielded qualitatively the same results (Table S4, Table S3).

Assessments of turgor loss point

Plants were grown in pots (13.8 cm diameter × 35.6 depth) with sand in the greenhouse under well-watered and regularly fertilized conditions until assessments of turgor loss point from November 2015 to February 2016. To validate the osmometer method that uses measurements of the osmotic potential at full turgor with an osmometer to assess turgor loss point, we measured osmotic potential at full turgor both with the osmometer method (Bartlett *et al.*, 2012a) and the pressure-volume curve method (Tyree & Hammel, 1972), and turgor loss point with the pressure-volume curve method.

Osmometer measurements of leaf osmotic potential at full turgor ($\pi_{o\text{-}osmo}$) were taken for all study species with 6 individuals per species. Plants were watered in the evening and fully rehydrated overnight in the dark to avoid transpiration at room temperature of about 20 °C. A healthy mature leaf from each individual was used to sample one disc (4-mm-diameter), avoiding major veins. The disc was wrapped in foil and submerged in liquid nitrogen for 2 minutes, then punctured 10-15 times, and sealed in an osmometer (VAPRO 5500, Wescor, Logan, UT; following Bartlett *et al.*, 2012a). Osmolality was repeatedly measured until equilibrium was reached (change between consecutive measurements < 2.5 mmol kg^{-1}). $\pi_{o\text{-}osmo}$ was converted from osmolality using the equation:

$$\pi_{o\text{-}osmo} = -RT \cdot \text{osmolality} = -0.0025 \cdot \text{osmolality}$$

where R is the universal gas constant, and T is 298.15 Kelvin (Sweeney & Beuchat, 1993).

Pressure-volume curves (P-V curves) were measured for a subset of 14 species (7 forbs, 7 grasses), using a bench drying method (Tyree & Hammel, 1972; Sack *et al.*, 2011) on three individuals per species to validate the osmometer assessments of turgor loss point. Plants were rehydrated overnight in the dark. One healthy mature leaf from each individual was cut in the morning. During bench drying, we repeatedly measured the leaf water potential (Ψ_{leaf}) and the

corresponding leaf weight. Ψ_{leaf} was measured with a pressure chamber (Model 1000 Pressure Chamber Instrument, PMS Instrument Company, USA) at Ψ_{leaf} intervals of approximately 0.2-0.3 MPa until Ψ_{leaf} reached -3.0 MPa (Sack *et al.*, 2011). Leaves were weighed on an analytical balance (to 0.00001g, Mettler AE163, Mettler-Toledo, Giessen, Germany). Subsequently, leaves were oven-dried to determine relative water content (RWC, (leaf weight – leaf dry weight)/(leaf saturated weight – leaf dry weight)). P-V curves ($1/\Psi_{leaf}$ versus RWC) were constructed after removing data points representing oversaturated symplastic water content (Kubiske & Abrams, 1991; Sack *et al.*, 2011). Turgor loss point ($\pi_{tlp\text{-}P\text{-}V}$, osmotic water potential at turgor loss), and leaf osmotic potential at full turgor ($\pi_{o\text{-}P\text{-}V}$) were calculated for each leaf from the P-V curves using standard methods (Tyree & Hammel, 1972).

Whole-plant drought survival and midday leaf water potential

To assess whole-plant drought survival and comparative midday leaf water potential (Ψ_{MD}) under drought conditions, we established a field drought experiment at the Ecological Botanical Garden of the University of Bayreuth, Germany. Plants were transplanted to 72 plots in a 20 cm grid with one individual per species being randomly assigned to each plot. All plots were covered with transparent rainout shelters. After an initial acclimation period under well-watered conditions, irrigation was discontinued for 10 weeks in half of the plots (3rd August – October 9th, 2015). Soil water potentials in drought plots declined sharply and started to reach -1.5 MPa, the permanent wilting point in agricultural plants, within 18 days of ceasing irrigation (Veihmeyer & Hendrickson, 1928; Manuscript 1). Plant drought survival was assessed weekly throughout the experiment based on living aboveground tissue as well as after potential resprouting from surviving underground organs in the next spring (April 2016). Survival ranking of the species remained consistent throughout, indicating that it was indipendent of drought duration and intensity. For details on the field experiment see Manuscript 1. We used the survival in April 2016 for further analyses.

Midday leaf water potential under drought was measured when most plants started wilting in eight randomly selected plots under the drought treatment (generally in 8 individuals per species). To ensure comparability across species and to avoid any bias introduced by sampling date, we sampled plants plot by plot, i.e. one individual of each species per day. Measurements were taken with leaf cutter psychrometers (Merrill Specialty Equipment, Logan, Utah, USA) and a PSYPRO™ water potential system (Wescor, Inc., Logan, Utah, USA). Samples were taken from 11:30 am to 12:30 pm from August 8th – 15th 2015. One leaf disk per individual

(diameter 6 mm) was sampled with leaf cutter psychrometers from the middle of a healthy, mature leaf avoiding major leaf veins, transported back to the laboratory, and equilibrated in a water bath at 25°C for 5 hours. Midday leaf water potentials were analyzed based on previously established calibration curves with 5 different levels of standard NaCl solutions for each sensor.

Statistical analyses

To assess if the osmotic potential at full turgor measured with an osmometer ($\pi_{o\text{-}osmo}$) is a suitable proxy for the turgor loss point in grassland species, we correlated $\pi_{o\text{-}P\text{-}V}$ and $\pi_{tlp\text{-}P\text{-}V}$ with $\pi_{o\text{-}osmo}$ across the 14 species measured in our study. To further assess the relation in herbaceous species, we compiled published data on $\pi_{o\text{-}P\text{-}V}$ and $\pi_{tlp\text{-}P\text{-}V}$ from traditional P-V curves (Bartlett *et al.*, 2012b; Gotsch *et al.*, 2015; Ocheltree *et al.*, 2016; Farrell *et al.*, 2017), and correlated $\pi_{o\text{-}P\text{-}V}$ with $\pi_{tlp\text{-}P\text{-}V}$. We used Standardized Major Axis (SMA) tests to test if the slopes and intercepts differed between forbs and grasses, and between the species in our study and previously published data. The regression equation between $\pi_{o\text{-}osmo}$ and $\pi_{tlp\text{-}P\text{-}V}$ from the 14 species in our study was subsequently used to calculate turgor loss point for all species based on osmometer measurements ($\pi_{tlp\text{-}osmo}$).

We tested differences of midday leaf water potentials under drought (Ψ_{MD}) and turgor loss points (π_{tlp}) among species and between life forms (forbs and grasses) using one-way ANOVAs for species and t-tests for life forms. Significance of the difference of whole-plant drought resistance (see below) between forbs and grasses was tested using a generalized linear model with binomial distributions (GLM).

To assess the association of turgor loss point with desiccation avoidance, the relation between Ψ_{MD} and $\pi_{tlp\text{-}osmo}$ was analyzed with a Pearson correlation, and we tested for a difference of this relation between forbs and grasses with a SMA test.

We analyzed the effects of Ψ_{MD} and $\pi_{tlp\text{-}osmo}$ on whole-plant drought resistance based on three different parameters: (a) drought survival as alive or dead (binary data), (b) percent survival in the drought treatment relative to the number of individuals at the start of the drought (% survival), and (c) the ratio of % survival in dry treatment plots relative to % survival in well-watered plots (survival ratio). We analyzed the effects of Ψ_{MD} or $\pi_{tlp\text{-}osmo}$ on drought survival (alive/dead), using a generalized linear model with binomial distributions (GLM) with drought survival as a response variable and Ψ_{MD} or $\pi_{tlp\text{-}osmo}$ as independent variable, and tested it with

a likelihood ratio test (Chi-square test). We included the effects of life form (forb and grass) and its interactions with Ψ_{MD} or $\pi_{tlp\text{-}osmo}$ as additional factors to test whether the effects of Ψ_{MD} and $\pi_{tlp\text{-}osmo}$ on whole-plant drought resistance differed between forbs and grasses. We also tested the effects of Ψ_{MD} and $\pi_{tlp\text{-}osmo}$ on % survival and survival ratio with Pearson's correlations. All models yielded qualitatively the same results (Table S4, except the relations of % survival with Ψ_{MD} and $\pi_{tlp\text{-}osmo}$ within forbs were marginally significant ($P < 0.1$)). The binary drought survival data (alive/dead) represents the basic and untransformed dataset and the distribution is accepted as non-normal, usually analyzed using GLM with binomial distribution (McCullagh, 1984). We therefore present the results for drought survival in the main text and provide the results for %survival and the ratio of %survival in the Table S4. However, to make figures easily visually understandable, we plotted %survival as responses and fitted the models with Pearson's correlations in the figures.

We further used a mediation test (Muthén *et al.*, 2016) to evaluate if the effects of turgor loss point on whole-plant drought resistance were indirectly mediated through midday leaf water potential, i.e. high turgor loss point allowing plants to maintain high midday leaf water potential during drought, leading to high drought survival (hypothesis 2), or if there was a direct effect of turgor loss point on whole-plant drought resistance. We assessed the mediation of Ψ_{MD} between $\pi_{tlp\text{-}osmo}$ and drought survival using Mplus with bootstrap = 10000 (Muthén *et al.*, 2016).

Standardized Major Axis tests were done using smatr3 packages in R (Warton *et al.*, 2012). The remaining analyses were performed in R using R base package (R Core Team, 2017).

Results

Validation of the osmometer method to assess turgor loss point in herbaceous species

Across 14 species including both forbs and grasses, turgor loss point assessed using P-V curves ($\pi_{tlp\text{-}P\text{-}V}$) was significantly and tightly correlated with osmotic potential at full turgor assessed from P-V curves ($\pi_{o\text{-}P\text{-}V}$, Fig. 1a). This relation was also significant within forbs or grasses (Fig. 1a). Consistently, a significant positive correlation between osmotic potential at full turgor and water potential at turgor loss assessed with P-V curves was found for data on 35 herbaceous species gathered from the literature ($r^2 = 0.79$, Fig. 1a, Bartlett *et al.*, 2012b; Gotsch *et al.*, 2015; Ocheltree *et al.*, 2016; Farrell *et al.*, 2017). Turgor loss point assessed with traditional P-

V curves was also significantly positively related to osmotic water potential at full turgor assessed with an osmometer ($\pi_{o\text{-}osmo}$, Fig. 1b). Again, relations remained significant within forbs and grasses separately (Fig. 1b). Slopes and intercepts did not differ between life forms (Standardized Major Axis tests, all *P* values > 0.88, Table S2). Turgor loss points were therefore consequently modelled from the osmometer measurements of osmotic potential at full turgor ($\pi_{tlp\text{-}osmo}$) based on the regression equation from our 14 species:

$$\pi_{tlp\text{-}osmo} = 0.645 \cdot \pi_{o\text{-}osmo} - 0.942 \qquad \text{Eqn 1}$$

Species midday leaf water potential, turgor loss point and drought survival

Midday leaf water potentials under drought (Ψ_{MD}) and turgor loss points ($\pi_{tlp\text{-}osmo}$) varied significantly across the 41 focal species (Table S3). Turgor loss points ranged from -2.30 ± 0.12 MPa to -1.49 ± 0.02 MPa (mean ± se) in our study and were well within the published data for herbaceous species under irrigated conditions, i.e. -2.96 MPa to -0.43 MPa (Fig. 1a, Bartlett *et al.*, 2012b; Farrell *et al.*, 2017). Forbs had significantly higher Ψ_{MD} and $\pi_{tlp\text{-}osmo}$ than grasses (t-test, P < 0.001, Fig. 2 a, Table S3). Between 42% and 100% of the individuals of focal species survived the experimental drought, with drought survival (alive/dead) being higher in forbs than in grasses (GLM, $P < 0.05$, Fig. 2c).

Midday leaf water potential under drought (Ψ_{MD}), indicating the species' integrated desiccation avoidance, was significantly positively related to the turgor loss point across all 41 species ($\pi_{tlp\text{-}osmo}$; $r^2 = 0.36$, $P < 0.001$, Fig. 3a; also see Table S4). Thus, species that lost turgor at high water potentials maintained high leaf water potentials under drought (effectively avoided desiccation). Turgor loss point had a strong effect on species drought survival (alive/dead), with species with higher turgor loss point exhibiting higher survival across all 41 species ($\chi^2 = 52.6$, $P < 0.001$, Table S4; also see Fig. 3b). Midday leaf water potential under drought (Ψ_{MD}) also had a strong positive effect on species drought survival (alive/dead) across all 41 species ($\chi^2 = 82.6$, $P < 0.001$, Table S4; also see Fig. 3c). All effects remained significant when forbs and grasses were analyzed separately (Table S4), and the effects did not differ significantly between forbs and grasses (Table S5).

The mediation test showed that effects of turgor loss point on drought survival (alive/dead) were predominantly indirect, i.e. through maintenance of high midday leaf water potential (*P*

< 0.001, Fig. 4). The total indirect effect size of high turgor loss point on species drought survival under drought was 7.66 and the nonsignificant direct effect size was 1.17.

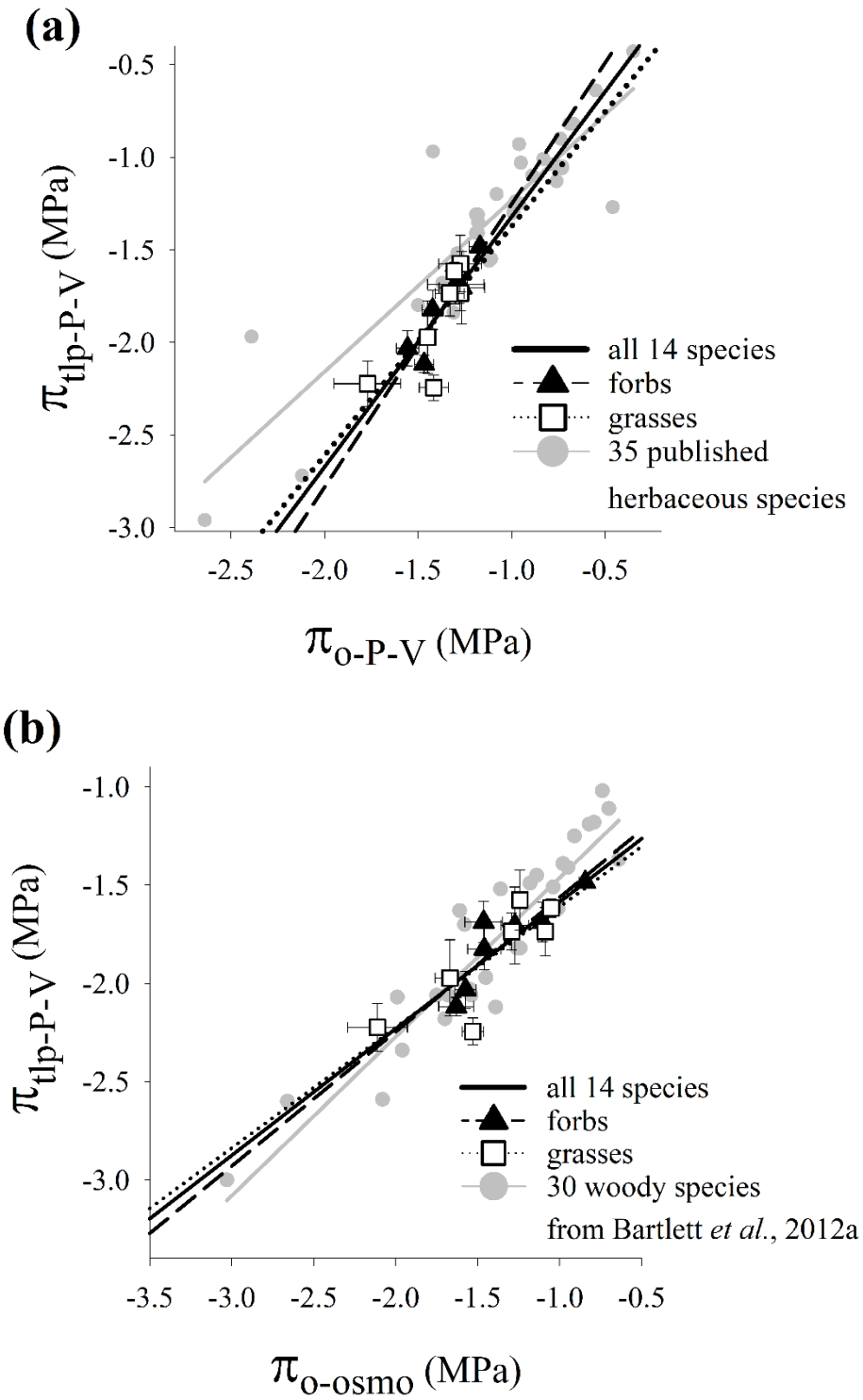

Fig. 1 Turgor loss point measured with the traditional P-V curve methods ($\pi_{tlp\text{-}P\text{-}V}$) was significantly related to osmotic water potential at full turgor (a) assessed with traditional P-V curves ($\pi_{o\text{-}P\text{-}V}$) across all pooled 14 grassland species in our study ($r^2 = 0.71$, $P < 0.001$) and within forbs ($r^2 = 0.87$, $P < 0.01$) and grasses ($r^2 = 0.61$, $P < 0.05$), and across 35 herbaceous species compiled from published data ($r^2 = 0.79$, $P < 0.001$; Bartlett *et al.*, 2012b; Gotsch *et*

al., 2015; Ocheltree *et al.*, 2016; Farrell *et al.*, 2017), and (b) assessed with an osmometer ($\pi_{o\text{-}osmo}$) across all pooled 14 grassland species in our study ($r^2 = 0.86$, $P < 0.001$) and within forbs ($r^2 = 0.77$, $P < 0.01$) and grasses ($r^2 = 0.69$, $P < 0.05$). In (b), for comparison we additionally included the relation across 30 woody species from Bartlett *et al.* (2012a). Data are species mean values ± standard errors with n = 3 individuals per species for 14 grassland species in our study, and species means for the data from published literature. In (b) the regression line for woody species (Bartlett *et al.*, 2012a, grey line) did not significantly differ from the one for all pooled 14 grassland species (black line) in our study. The joint regression equation is: $\pi_{tlp\text{-}osmo} = 0.786 \cdot \pi_{o\text{-}osmo} - 0.705$, n = 44 species.

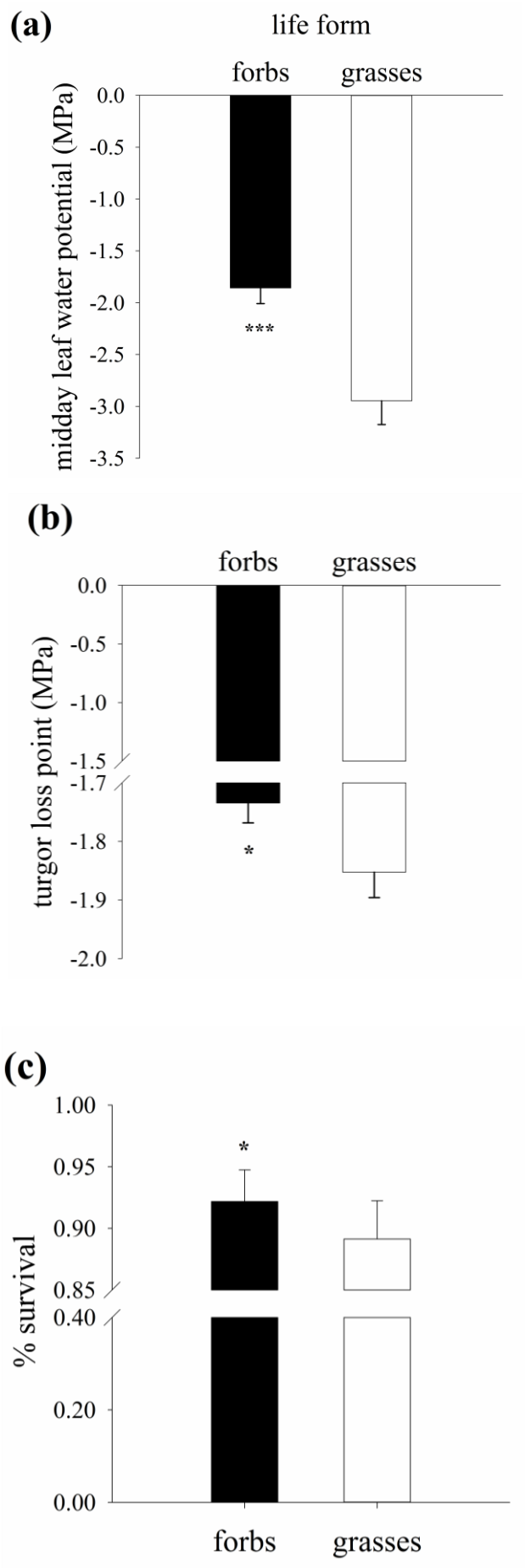
(a)
life form
forbs
grasses
midday leaf water potential (MPa)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
***
(b)
forbs
grasses
turgor loss point (MPa)
0.0
-0.5
-1.0
-1.5
-1.7
-1.8
-1.9
-2.0
*
(c)
% survival
1.00
0.95
0.90
0.85
0.40
0.20
0.00
*
forbs
grasses

Fig. 2 Comparisons between forbs and grasses of (a) midday leaf water potentials under drought (Ψ_{MD}), (b) turgor loss points ($\pi_{tlp\text{-}osmo}$) and (c) species survival under drought. The figure shows % survival for visual understandability, while the test of the difference of drought survival (alive/dead) between forbs and grasses presented in the text is based on GLM with binomial distribution. n = 20 for forbs and n = 21 for grasses. Significance of differences is given as *** $P < 0.001$, * $P < 0.05$.

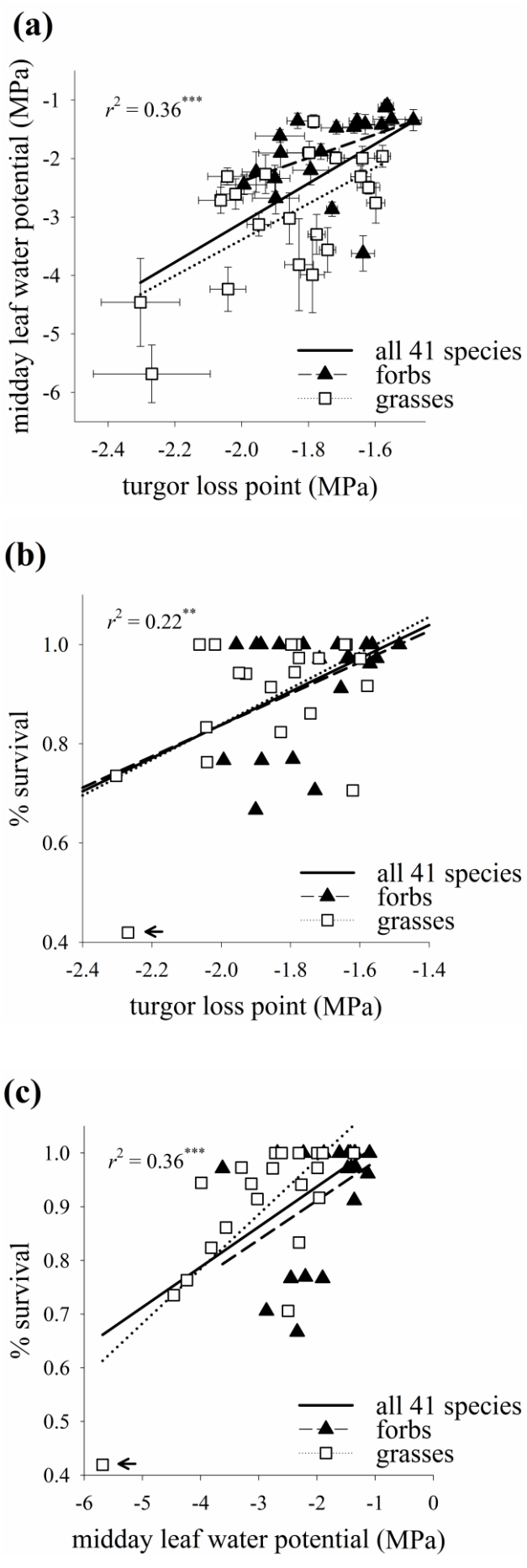
(a)
midday leaf water potential (MPa)
$r^2 = 0.36^{***}$
all 41 species
forbs
grasses
turgor loss point (MPa)
(b)
% survival
$r^2 = 0.22^{**}$
all 41 species
forbs
grasses
turgor loss point (MPa)
(c)
% survival
$r^2 = 0.36^{***}$
all 41 species
forbs
grasses
midday leaf water potential (MPa)

Fig. 3 Turgor loss point ($\pi_{tlp\text{-}osmo}$) was positively related to (a) midday leaf water potential under drought (Ψ_{MD}), and (b) species survival under drought. Higher Ψ_{MD} also was positively related to species survival under drought (c). Data points are averages ± standard errors for all 41 species. All relations were also significant within forbs or grasses, and relations stayed significant when the outlier (arrow) was excluded (for details see Table S4). The figure shows % survival fitted with Pearson's correlations for visual understandability, while the relations of $\pi_{tlp\text{-}osmo}$ and Ψ_{MD} with drought survival (alive/dead) presented in the text are based on GLM with binomial distribution. Significance is given as *** $P < 0.001$, ** $P < 0.01$.

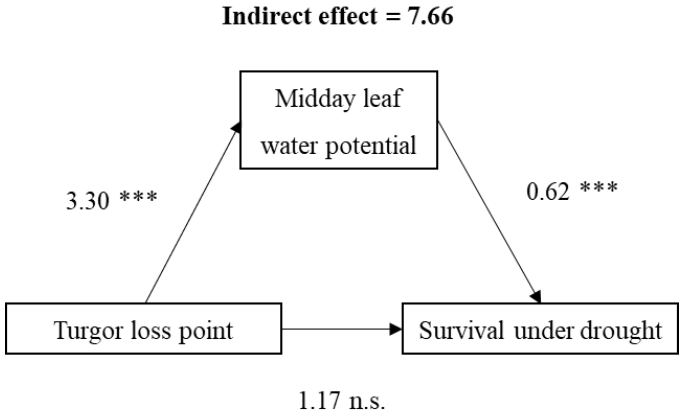

Fig. 4 Schematic representation of the results of the mediation test for the direct and indirect effect of turgor loss point on drought survival (alive/dead). Numbers adjacent to arrows indicate the effect size and the associated *P* value. ***: *P* value < 0.001, n.s.: *P* value > 0.05.

Discussion

Turgor loss point, desiccation avoidance and drought survival in grassland species

The positive relation between turgor loss point and drought survival we clearly showed that in grassland species a *high* turgor loss point promotes high drought survival. A high turgor loss point may promote drought survival through enabling plants to lose turgor and close stomata earlier during drought, and to maintain high water content (i.e. avoid desiccation). We also showed that the midday leaf water potential mediated the positive effect of turgor loss point on drought survival, indicating that desiccation avoidance mechanisms are driving differential whole-plant drought resistance of grassland species rather than desiccation tolerance mechanisms. Both forbs and grasses exhibited a strategy of desiccation avoidance, as indicated by the consistent positive relations among turgor loss point, midday leaf water potential and drought survival in both life forms. Forbs had higher turgor loss points and maintained higher midday leaf potentials under drought compared to grasses, implying forbs being more effective in avoiding desiccation, and thereby had higher drought survival than grasses (Fig. 2, Fig. 3). Although this mechanism has been recognized, it has received little attention (Bartlett *et al.*, 2012b, references within). A meta-analysis focusing on woody species across biomes found no support for a high turgor loss point or a high leaf water content leading to plant drought resistance (Bartlett *et al.*, 2012b), contrary to our results for grassland species.

Turgor loss at high leaf water potentials can allow plants to avoid desiccation through several mechanisms that minimize leaf water loss under decreasing water availability. Firstly, turgor loss triggers the biosynthesis of the phytohormone abscisic acid (ABA), which leads to stomatal closure even at high water potentials (McAdam & Brodribb, 2016). A mechanistic linkage between turgor loss point and stomatal closure is supported by a positive relation across 7 herbaceous species (reanalysed from Farrell *et al.*, 2017), i.e. species with high turgor loss point close stomata early under decreasing leaf water potentials. Secondly, in many species turgor loss induces leaf rolling or folding and vertical leaf orientation (Turner & Begg, 1981), which maximizes boundary layer resistance and minimizes leaf-to-air water vapor deficit. Additionally, turgor loss leads to the shrinking of cuticle waxes, which reduces cuticular conductance (i.e. cuticle permeability to water vapor), the main path of plant water loss after stomatal closure (Boyer, 2015). These mechanisms may individually or in combination contribute that a high turgor loss point minimizes leaf water loss, and thus allows grassland species to maintain high leaf water potentials and to high survival under drought. Pairwise

comparisons of drought responses in herbaceous species with different turgor loss points are consistent with our finding that high turgor loss point leads to high drought survival in herbaceous grassland species (Barnes, 1985; Torrecillas *et al.*, 1995; Braatne & Bliss, 1999; Holloway-Phillips & Brodribb, 2011).

Additional mechanisms of desiccation avoidance have consistently been found to be important for the drought performance of grassland species or for their distribution. Low stomatal conductance under non-stressed conditions and stomatal closure at high water potentials (i.e. 'earlier' in a drought) were related to maintenance of productivity or occurrence in dry habitats in some studies, supporting the role of minimizing water loss (Thomas, 1986; Belluau & Shipley, 2017; Konings *et al.*, 2017). In contrary, two other studies showed a trend towards species with high water potentials at stomatal closure occuring in wetter sites (Tucker *et al.*, 2011; Craine *et al.*, 2013), although relevant data supporting desiccation tolerance mechanisms for grassland species remain scare. Xylem embolism resistance, one of the most important desiccation tolerance traits in woody plants (Anderegg *et al.*, 2016; O'Brian *et al.*, 2017) and pit membrane thickness were unrelated to species drought survival in our study (data analyzed from Lens *et al.*, 2016 for 13 and 5 species, respectively). Consistently, xylem embolism resistance was not related to habitat water availability across 8 C4 grasses (Ocheltree *et al.*, 2016). In conclusion, a decisive role of mechanisms of desiccation avoidance for differential drought resistance of grassland species is strongly supported by our own as well as previous results, while a relevance of mechanisms of desiccation tolerance is not supported.

Contrasting mechanisms of drought resistance in grassland and woody species

The trend we found in herbaceous grassland species, i.e. a high turgor loss point promoting drought survival, was opposite to studies on woody species (Lenz *et al.*, 2006; Mitchell *et al.*, 2008; Bartlett *et al.*, 2012b; Maréchaux *et al.*, 2015; Zhu *et al.*, 2017). These results imply fundamentally different strategies of drought resistance in herbaceous and woody species.

In woody species, a *low* (more negative) turgor loss point confers high drought resistance as a mechanism of *desiccation tolerance* by allowing the leaf to remain turgid despite of decreasing leaf water potentials (Ψ_{leaf}), and therefore maintain key physiological functions such as cell expansion during growth (Cheung *et al.*, 1975; Frensch & Hsiao, 1994; Velazquez-Marquez *et al.*, 2015), stomatal openness for photosynthetic CO_2 assimilation (Brodribb *et al.*, 2003), and whole leaf hydraulic conductivity (Bartlett *et al.*, 2012b; Scoffoni *et al.*, 2017).

Mechanisms of desiccation tolerance generally dominate in woody species: high resistance to xylem embolism in leaves, stems and roots, low lethal leaf water potentials and low water potentials at stomatal closure have previously been linked to drought survival across woody species (Kursar *et al.*, 2009; Urli *et al.*, 2013; Anderegg *et al.*, 2015; Anderegg *et al.*, 2016; Zhu *et al.*, 2017; Johnson *et al.*, 2018). In turn, a low turgor loss point has been shown to be associated with these physiological mechanisms of desiccation tolerance (Bartlett *et al.*, 2016). On the other hand, maximum rooting depth, a prominent trait of drought avoidance, was unrelated to drought mortality across woody species (Anderegg *et al.*, 2016). In summary, for woody species there is plenty of evidence - including from assessments of turgor loss points - that mechanisms of desiccation tolerance are important for drought survival, while a decisive role of mechanisms of desiccation avoidance is not supported. This is in stark contrast to our and other findings for grassland species (see above).

The distinct strategies of drought resistance in grassland and woody species are likely due to their fundamental differences in biomass allocation patterns and their morphological and physiological traits. Temperate grassland species overall have much higher root:shoot ratios (about 10 fold for comparion of temperate grasslands with temperate, subtropical and tropical woodlands; Mokany *et al.*, 2006) and smaller leaf areas than woody species (Nolf *et al.*, 2016). This leads to higher water uptake capacity relative to water demand for transpiration in grassland species, which benefits desiccation avoidance. Second, adults of temperate grassland species are much smaller than the vast majority of woody species (shrubs and trees) and therefore have a shorter water transport path. This renders the gravitational component of water potential negligible, and strongly reduces the length dependent hydraulic resistance of the xylem in grassland species, while these components can considerably lower leaf water potentials in tall trees (Tyree, 2007). Lastly, stomatal closure under low humidity or water availability is more sensitive to ABA in herbaceous species than woody species (McAdam & Brodribb, 2015). The stomata of herbaceous species are therefore more responsive to decreasing water potentials, which contributes to avoiding water loss. The mechanisms above facilitate in grassland species the maintenance of high leaf water potentials, i.e. desiccation avoidance, and may release selection pressure for tissue tolerance of low water potentials, i.e. desiccation tolerance, relative to woody species.

Conclusions

Our study indicated that turgor loss point is driving differential drought resistance of temperate grassland species through functioning as a mechanism of desiccation avoidance, which was contrary to woody species, a result that underlines the differences of strategies to cope with drought between temperate grassland species and woody species. Our results highlight the need to directly link functional traits with whole-plant drought survival for characterizing drought resistance strategies in different plant life forms, and for predicting plant drought responses based on trait assessments. Turgor loss point, which can be efficiently assessed with the 'osmometer method', was shown to be a promising trait to advance projections of drought survival in temperate grassland species. Nevertheless, the relation was relatively weak and the predictive power therefore remains limited. Identifying additional traits relevant for drought survival in grassland species has the potential to improve predictions of which species will lose or win, and how community composition and ecosystem functions will be affected by altered drought regimes under global climate change.

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Author contribution

B.M.J.E. and S.S. designed the research, S.S., E.J., J.G. performed the experiments, S.S. and B.M.J.E. analyzed the data, S.S., E.J. and B.M.J.E. wrote the manuscript, all co-authors revised the manuscript.

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Supporting information

Text S1 Discussion on turgor loss point assessment

We validated for herbaceous species the 'osmometer method' (Bartlett *et al.*, 2012a) to assess the turgor loss point from measurements of osmotic water potential at full turgor (Fig. 1). The 'osmometer method' which allows to rapidly assess turgor loss points thus can be extended from woody to non-woody species. The relations did not differ between forbs and grasses, or between herbaceous and woody species (this study vs. Bartlett *et al.*, 2012a, and all herbaceous species in this study and previous studies vs. all woody species in Bartlett *et al.*, 2012b; Table S2), suggesting that despite the fundamental differences in the strategy to cope with drought a common equation is suitable for woody and herbaceous species, including for new taxa, to assess turgor loss point from measurements of osmotic potential at full turgor with an osmometer. The data from the 14 herbaceous species in our study and the 30 woody species from Bartlett *et al.* (2012a) could therefore be included in a joint regression (see Fig. 1 for equation). The 'osmometer method' will greatly facilitate rapid quantification of drought resistance in grasslands, similar to woody species (Bartlett *et al.*, 2012a).

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Table S1 List of the 43 temperate grassland species in our study with family and life form.

Species	Family	Life form
Achillea millefolium L.	Asteraceae	Forb
Agrostis capillaris L.	Poaceae	Grass
Agrostis stolonifera L.	Poaceae	Grass
Alopecurus pratensis L.	Poaceae	Grass
Anthoxanthum odoratum L.	Poaceae	Grass
Arrhenatherum elatius (L.) P.Beauv. ex J.Presl & C.Presl.	Poaceae	Grass
Brachypodium pinnatum (L.) P.Beauv.	Poaceae	Grass
Briza media L.	Poaceae	Grass
Bromus hordeaceus L.	Poaceae	Grass
Centaurea jacea L.	Asteraceae	Forb
Cerastium holosteoides Fr.	Caryophyllaceae	Forb
Cirsium oleraceum (L.) Scop.	Asteraceae	Forb
Crepis biennis Lapeyr.	Asteraceae	Forb
Dactylis glomerata L.	Poaceae	Grass
Daucus carota L.	Apiaceae	Forb
Elymus repens (L.) Gould	Poaceae	Grass
Festuca guestfalica Boenn. ex Rchb.	Poaceae	Grass
Festuca ovina L.	Poaceae	Grass
Festuca pratensis Huds.	Poaceae	Grass
Festuca rubra L.	Poaceae	Grass
Galium mollugo L.	Rubiaceae	Forb
Helictotrichon pubescens (Huds.) Schult. & Schult.f.	Poaceae	Grass
Holcus lanatus L.	Poaceae	Grass
Lathyrus pratensis L.	Fabaceae	Forb
Leontodon autumnalis L.	Asteraceae	Forb
Leucanthemum vulgare (Vaill.) Lam.	Asteraceae	Forb
Lolium perenne L.	Poaceae	Grass
Lotus corniculatus L.	Fabaceae	Forb
Medicago lupulina L.	Fabaceae	Forb
Phleum pretense L.	Poaceae	Grass

Plantago lanceolata L.	Plantaginaceae	Forb
Poa pratensis L.	Poaceae	Grass
Poa trivialis L.	Poaceae	Grass
Prunella vulgaris L.	Lamiaceae	Forb
Ranunculus acris L.	Ranunculaceae	Forb
Ranunculus bulbosus L.	Ranunculaceae	Forb
Ranunculus repens L.	Ranunculaceae	Forb
Rumex acetosa L.	Polygonaceae	Forb
Rumex crispus L.	Polygonaceae	Forb
Taraxacum officinale (L.) Weber ex F.H.Wigg.	Asteraceae	Forb
Trifolium repens L.	Fabaceae	Forb
Trisetum flavescents (L.) P. Beauv.	Poaceae	Grass
Vicia cracca L.	Fabaceae	Forb

Table S2 Results of Standard Major Axis (SMA) tests for differences of relations in testing the 'osmometer method' (Figure 1). A. comparison between grasses and forbs in this study, B. comparison between 14 grassland species in this study and 30 woody species in Bartlett *et al.* (2012a), C. comparison between herbaceous species (14 grassland species in this study and published data for 35 herbaceous species, Bartlett *et al.*, 2012b; Gotsch *et al.*, 2015; Ocheltree *et al.*, 2016; Farrell *et al.*, 2017) and predominant woody species (221 species, Bartlett *et al.*, 2012b). Results are for the relations of (a) turgor loss point vs. osmotic potential at full turgor measured with the traditional P-V curve method ($\pi_{tlp\text{-}P\text{-}V}$ vs $\pi_{o\text{-}P\text{-}V,}$ respectively, Tyree & Hammel, 1972), (b) turgor loss point from pressure volume curves vs. osmotic potential at full turgor assessed with an osmometer ($\pi_{o\text{-}osmo}$, Bartlett *et al.*, 2012a), and (c) osmotic potential at full turgor assessed from pressure volume curves vs. with an osmometer.

Parameter relations	A. grasses vs. forbs		B. grassland vs. woody		C. herbaceous vs. woody	
	slope	intercept	slope	intercept	slope	intercept
(a) $\pi_{tlp\text{-}P\text{-}V} \sim \pi_{o\text{-}P\text{-}V}$	0.92	0.98	0.37	0.90	0.11	0.67
(b) $\pi_{tlp\text{-}P\text{-}V} \sim \pi_{o\text{-}osmo}$	0.88	0.89	0.42	0.24		
(c) $\pi_{o\text{-}P\text{-}V} \sim \pi_{o\text{-}osmo}$	0.95	0.84	0.06	0.30		

Given are *P* values for differences of slopes and intercepts between the respective different groups. Note that none of the groups differ significantly from each other.

Table S3 Differences among species or between life forms in midday leaf water potential under drought (Ψ_{MD}), turgor loss point ($\pi_{tlp\text{-}osmo}$) and drought survival (alive/dead, compare Fig. 2) for datasets (a) including or (b) excluding outlier *Poa trivialis* L., or (c) including all 43 initial species (i.e. including *Medicago lupulina* L. and *Trifolium repens* L. which had < 70% survival even under well-watered conditions, compare table S4 and methods).

Traits	Groups	(a) 41 species used in this study	(b) 40 species excluding *outlier*	(c) 43 all initial species
midday leaf water potential	Species	10.74***	8.90***	10.45***
	(*F* value)	(40, 256)	(39, 260)	(42, 270)
	Life form	-3.98***	3.90***	-4.11***
	(*t* value)	(34.28)	(35.90)	(33.10)
turgor loss point	Species	8.37***	13.89***	9.22***
	(*F* value)	(40, 217)	(39, 207)	(42, 238)
	Life form	2.13*	1.86(*)	2.28*
	(*t* value)	(37.15)	(36.12)	(37.10)
drought survival	Life form	*5.24**	0.92 ns	1.18 ns
	(χ^2 value)	*(1)*	(1)	(1)

Given are *F* values (one-way ANOVA for species differences), *t* values (t-tests for life form differences, grasses vs. forbs) or χ^2 values (GLM with binomial distribution for life form differences in drought survival (alive/dead)), and associated degrees of freedom in parentheses. The change for drought survival between significant vs. non-significant results among datasets is given in italics. (*), $P < 0.1$ (marginally significant); *, $P < 0.05$; ***, $P < 0.001$; ns, $P > 0.1$ (not significant).

Table S4 Relations of midday leaf water potential under drought (Ψ_{MD}), turgor loss point ($\pi_{tlp\text{-}osmo}$) and three different parameters of drought resistance (drought survival, % survival and survival ratio, see methods) for datasets (a) across the 41 main species (compare main text), (b) excluding the outlier *Poa trivialis* L. (40 species, compare Fig. 3), or (c) including all 43 initial species (i.e. including *Medicago lupulina* L. and *Trifolium repens* L.). Relations are given for forbs and grasses combined and separated.

Parameter relations	(a) 41 species used in this study	(b) 40 species excluding *outlier*	(c) 43 all initial species
midday leaf water potential ~ turgor loss point	r^2	r^2	r^2
across all species	0.36 ***	0.29***	0.38***
within forbs	0.21*	0.21*	0.23*
within grasses	0.34**	0.23*	0.34**
drought survival ~ midday leaf water potential	χ^2	χ^2	χ^2
across all species	82.6***	36.0***	38.9***
within forbs	18.0***	18.0***	6.1*
within grasses	67.7***	23.9***	67.7***
% survival ~ midday leaf water potential	r^2	r^2	r^2
across all species	0.36***	0.18**	0.10*
within forbs	0.18 (*)	0.18 (*)	*0.02 ns*
within grasses	0.56***	0.31*	0.56***
survival ratio ~ midday leaf water potential	r^2	r^2	r^2
across all species	0.38***	0.20**	0.13*
within forbs	0.20*	0.20*	*0.03 ns*
within grasses	0.58***	0.34**	0.58***

drought survival ~ turgor loss point	χ^2	χ^2	χ^2
across all species	52.6***	23.1***	17.3***
within forbs	19.9***	19.9***	*1.1 ns*
within grasses	29.0***	7.4**	29.0***
% survival ~ turgor loss point	r^2	r^2	r^2
across all species	0.22**	0.12*	*0.04 ns*
within forbs	0.17 (*)	0.17 (*)	*0.003 ns*
within grasses	0.23*	*0.10 ns*	0.23*
survival ratio ~ turgor loss point	r^2	r^2	r^2
across all species	0.20**	0.11*	*0.05 ns*
within forbs	0.20*	0.20*	*0.006 ns*
within grasses	0.18 (*)	*0.06 ns*	0.18 (*)

Given are r^2 or χ^2 values. Note that relations stay qualitatively the same regardless of the drought resistance parameter or dataset used. Changes between significant vs. non-significant relations between drought resistance parameters are underlined, and those between datasets are given in italics. (*), $P < 0.1$ (marginally significant); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, $P > 0.1$ (not significant).

Table S5 Differences between grasses and forbs in relations of midday leaf water potential under drought (Ψ_{MD}), turgor loss point ($\pi_{tlp\text{-}osmo}$) and three different parameters for drought resistance (drought survival, % survival and survival ratio, compare Fig. 3).

Relations	41 species used in this study	
	slope	intercept
midday leaf water potential ~ turgor loss point	0.56	0.07
drought survival ~ midday leaf water potential	0.18	0.79
% survival ~ midday leaf water potential	0.40	0.001
survival ratio ~ midday leaf water potential	0.31	0.002
drought survival ~ turgor loss point	0.25	0.24
% survival ~ turgor loss point	0.84	0.22
survival ratio ~ turgor loss point	0.73	0.24

Given are the *P* values for differences of slopes and intercepts tested with GLM with binomial distribution for relations regarding drought survival (alive/dead) and SMA for the remaining relations.

Manuscript 3: Root traits and turgor loss point explain differential drought resistance in temperate grassland species

Root traits and turgor loss point explain differential drought resistance in temperate grassland species

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Abstract

1. Drought induces plants mortality, reduces productivity and influences biodiversity in grasslands worldwide. Various traits that maximize water uptake and minimize water loss are hypothesized or known to influence species drought resistance. However, rigorous evaluations of their importance and predictive capacities for drought resistance are missing, especially for grassland species. This impedes our understanding of mechanisms of drought resistance and predictions of grassland dynamics under climate change.
2. We measured 15 root traits regarding morphology, allocation and phenotypic plasticity in response to drought as well as turgor loss point for 41 common temperate grassland species including 20 forbs and 21 grasses. We directly linked traits to species comparatively assessed midday leaf water potentials under drought, characterizing desiccation avoidance, and whole-plant drought resistance in terms of survival. We evaluated trait importance and the predictive power of various trait combinations for drought resistance.
3. Root traits and turgor loss point were related to whole-plant drought resistance, while they differed in their trait importance. Root traits were more important for drought resistance than turgor loss point across all species as well as within forbs and within grasses, underlining the importance of maximizing water uptake in grassland species. Among all root traits, rooting depth and root mass had overall the highest importance for drought resistance for both forbs and grasses, while root diameter only for forbs and phenotypic plasticity of root traits with minor importance. Forbs and grasses differed in their functional traits and trait importance for drought resistance, supporting the treatment of forbs and grasses as different functional groups. Combinations of relatively easily assessed traits under irrigated conditions had high predictive power for drought resistance for both groups ($r^2 \geq 56\%$), i.e. root morphological traits (root diameter, root tissue density and specific root length) for forbs and root allocation traits (root mass, rooting depth and root depth distribution) for grasses.
4. Our results highlighted the importance of root systems for promoting drought resistance in grassland species. The combinations of important root traits may contribute to improving projections of the dynamics of temperate grasslands under global climate change.

Key words:

Climate change, Grasses, Forbs, Rooting depth, Whole-plant drought resistance, Drought survival, Phenotypic plasticity

Introduction

Grasslands are one of the most widespread ecosystems covering one-fifth of the land surface and store one-third of terrestrial carbon (Scurlock & Hall 1998). They also provide important goods and are crucial for the maintenance of biodiversity (Gibson 2009). However, extreme climate events such as drought induce plants mortality and species loss, lead to decrease in grassland productivity and influence species composition globally (Weaver 1968; Tilman & El Haddi 1992; Debinski *et al.* 2010; Tucker, Craine & Nippert 2011; Yang *et al.* 2011). Under climate change scenarios drought intensity and frequency are projected to increase (IPCC 2014), even during the growing season (Orth, Zscheischler & Seneviratne 2016; Schlaepfer *et al.* 2017) enhancing drought effects on grasslands. Drought resistance in grassland species varies across species (Buckland *et al.* 1997; Tucker, Craine & Nippert 2011; Craine *et al.* 2012; Lens *et al.* 2016; Manuscript 1), and the effects of drought on grasslands, therefore, depend on species composition (Pfisterer & Schmid 2002; Isbell *et al.* 2015; Wagg *et al.* 2017). A thorough understanding of mechanisms underlying differential species drought resistance is necessary for the projections of dynamics of grassland under climate change.

Plants can resist drought stress by avoiding low leaf water potentials through maximizing water uptake and water storage and minimizing water loss, i.e. desiccation avoidance, or by tolerating low water potentials and maintaining water transport and photosynthesis despite of low water potentials, i.e. desiccation tolerance (Levitt 1972; Vilagrosa *et al.* 2012; Comita & Engelbrecht 2014). The effectiveness of plants to avoid desiccation can be assessed as the midday leaf water potentials under drought (MWP), which integrates the various mechanisms at the root, stem and leaf level (Levitt 1972; Vilagrosa *et al.* 2012; Comita & Engelbrecht 2014; Choat *et al.* 2018). Across temperate grassland species, the effectiveness of desiccation avoidance varies strongly, and midday water potentials under drought were positively related to comparative whole-plant drought resistance across 41 species (Manuscript 2). These findings, which were consistent with several previous studies (Garwood & Sinclair 1979; Buckland *et al.* 1997; Perez-Ramos *et al.* 2013; Zwicke *et al.* 2015; Belluau & Shipley 2017; Konings, Williams & Gentine 2017), provided conclusive evidence that differences in desiccation avoidance are important in driving the differential drought resistance of temperate grassland plants.

Turgor loss point (TLP), the leaf water potential at which leaf cells lose turgor, is a crucial mechanism of desiccation avoidance involved in controlling leaf level water loss. A high turgor

loss point can minimize water loss and contribute to high midday leaf water potentials under drought through several mechanisms, including triggering the biosynthesis of ABA and stomatal closure (Brodribb & Holbrook 2003; McAdam & Brodribb 2016), leaf rolling or folding and vertical leaf orientation (Turner & Begg 1981) and shrinking of cuticle waxes which reduces cuticular conductance (Boyer 2015). Turgor loss point was positively related to desiccation avoidance and whole-plant drought resistance in grassland species (Manuscript 2), indicating that it is a key trait. However, turgor loss point only explained part of the large variation across species in midday leaf water potentials under drought (36%) and whole-plant drought resistance (20%, Manuscript 2). Additional mechanisms, therefore, must also contribute to the observed variation of drought resistance. Water uptake through roots influences plant desiccation avoidance and therefore may also contribute to drought resistance in grassland species.

Roots vary in morphology, allocation and phenotypic plasticity among grassland species (Canadell *et al.* 1996; Jackson *et al.* 1996; Bardgett, Mommer & De Vries 2014; Barkaoui, Roumet & Volaire 2016; Larson & Funk 2016; Guderle *et al.* 2018). Various root traits have been hypothesized or shown to be important for maintenance of high leaf water potentials under drought and/or promoting plants drought resistance by maximizing soil water uptake (Ekanayake *et al.* 1985; Canadell *et al.* 1996; Barkaoui, Roumet & Volaire 2016; Freschet & Roumet 2017; Guderle *et al.* 2018). For example, high specific root length (SRL), indicating thin roots and/or low root tissue density, should lead to greater absorptive root length per unit biomass and may enable plants to efficiently increase root volume and surface to explore soil water (Bardgett, Mommer & De Vries 2014). High root mass ratio, indicating high biomass allocation to roots that take up water relative to transpirational leaf area should improve survival under drought (Comas *et al.* 2013). High rooting depth and high biomass allocation to deep roots allowed plants to use deep soil water and thus to maintain high leaf water potentials or water contents when surface soil dried out under drought (Buckland *et al.* 1997; Perez-Ramos *et al.* 2013; Barkaoui, Roumet & Volaire 2016; Guderle *et al.* 2018). Additionally, roots are phenotypically plastic in response to water availability with respect to root allocation and morphology, enabling plants to increase their water uptake during temporal drought stress (Hoeppner & Dukes 2012; Larson & Funk 2016). These root traits may all influence species drought resistance but to different degrees.

Several studies have tested importance of either turgor loss point or root traits for species differential drought resistance, and the use of either to predict drought resistance (Molyneux & Davies 1983; Tucker, Craine & Nippert 2011; Ocheltree, Nippert & Prasad 2016; Farrell, Szota & Arndt 2017; Belluau & Shipley 2018) because experimental assessments of species drought resistance need tremendous time and labor while the measurement of traits are relative easier (Manuscript 1). By relating traits to habitat water availability or to shoot growth or aboveground biomass production under drought conditions, these studies found that associations of either turgor loss point or root traits with drought resistance were weak and their predictive power was generally low (Molyneux & Davies 1983; Tucker, Craine & Nippert 2011; Ocheltree, Nippert & Prasad 2016; Farrell, Szota & Arndt 2017; Belluau & Shipley 2018). One reason for this may be that the linkages of species growth under drought and distribution along soil water availability with drought resistance are loose. Reduced growth or leaf mortality has been shown to be positively related to high whole-plant drought resistance, i.e. negative relations between growth under drought and drought resistance, suggesting that it may promote drought resistance by reducing transpirational area (Volaire, Thomas & Lelievre 1998; Volaire & Norton 2006; Manuscript 1), while it was also been reported to precede drought mortality, i.e. positive relations (Rivero *et al.* 2007; Zwicke *et al.* 2015). Other factors may additionally shape species distribution along moisture gradients besides drought, such as grazing, nutrient, light and biotic interactions (Proulx & Mazumder 1998; Hacker *et al.* 2006; Maron & Crone 2006; Borer *et al.* 2014; Breitschwerdt, Jandt & Bruelheide 2018). Supportively, in Manuscript 1 we found that although drought sensitive species were excluded from dry habitat, drought resistance species were associated with a wide range of moisture gradients. Therefore, to rigorously evaluate the importance of traits and their predictive power for species drought resistance, we need to directly relate traits to comparative assessments of whole-plant drought resistance (e.g. Manuscript 2). Assessment of whole-plant drought resistance under experimental drought conditions allow us to comparatively quantify the effects of drought on multiple species by inducing only drought stress while eliminating effects of other confounding factors, such as light, nutrients, interactions among plants or pot effects that may influence performances of individual species (e.g. Engelbrecht and Kursar 2003). However, such assessment remained scarce. The few studies in grassland species tested only a limited number of species (4-7 species; Volaire 2008; Perez-Ramos *et al.* 2013; Zwicke *et al.* 2015). In manuscript 1, we experimentally assessed drought resistance in 41 grassland species which now allows to directly relate traits to species comparative drought resistance and to assess their importance and predictive power.

Further, the importance of turgor loss point and root traits for drought resistance likely differs. Buckland *et al.* (1997) found that species with deep roots maintained leaf turgor and higher water content than those with low rooting depth, which suggests that root access to deep soil was more important than decreasing water loss at the leaf level. Meanwhile, strong control of water status through avoiding leaf level water loss is suggested by findings that species with shallow roots can still maintain high leaf water potential and water content under drought (Garwood & Sinclair 1979; Buckland *et al.* 1997). The role of different mechanisms of desiccation avoidance can also differ among life forms (Skelton, West & Dawson 2015), even within grassland species (Manuscript 2). For example, forbs lost turgor and closed stomata earlier than grasses as leaf water potentials declined (Tucker, Craine & Nippert 2011; Manuscript 2). Grasses increased root biomass allocation to deep soils under drought whereas forbs did not respond in their root depth distributions (Skinner & Comas 2010). However, a quantification of the relative importance of turgor loss point and root traits for desiccation avoidance and drought resistance across grassland species, and a comparison between forbs and grasses are still missing. This largely hinders our understanding of mechanisms of drought resistance and diminishes our predictive power of the consequences of climate change in shifts of grassland life form composition and ecosystem functions and services.

In this study, we analyzed turgor loss point and 15 root traits of 41 common temperate grassland species including both forbs and grasses, and linked them directly to species comparative midday leaf water potentials under drought, indicative of the effectiveness of their desiccation avoidance (Manuscript 2), and whole-plant drought resistance assessed in a common garden drought experiment (Manuscript 1). All traits are known or hypothesized to influence plant water relations, and the respective expected relations to desiccation avoidance and whole-plant drought resistance are indicated in Table 1. We aimed to elucidate (1) the relative importance of turgor loss point and individual root traits for the differential desiccation avoidance and drought resistance of grassland species; (2) how their importance varies between forbs and grasses; and (3) the predictive power of various trait combinations for drought resistance.

Materials and Methods

41 common temperate grassland species (20 forbs and 21 grasses, Table S1) were chosen according to (1) their high abundance and frequency in 150 long-term grassland plots (4m ×

4m) in Biodiversity Exploratories in three areas across Germany (Schorfheide-Chorin, Hainich-Dün and Schwäbische Alb; Fischer *et al.* 2010), (2) their associations with a wide range of soil moisture (Ellenberg indicator values for soil moisture from 3 to 7, Ellenberg *et al*. 1991), and (3) being perennial and C3, the dominant life history strategy and photosynthetic pathway in European temperate grasslands (Ellenberg *et al*. 1991; Collatz, Berry & Clark 1998). Seeds were purchased from commercial seed suppliers (Rieger-Hofmann GmbH and Saaten Zeller, Germany) and were germinated in a greenhouse in mid-March 2015. After three months of growth, plants were transplanted to a greenhouse and a common garden.

In the greenhouse experiment, plants were growing in deep pots (7 cm diameter × 36 cm depth). Plants were well-watered every day and fertilized with a full fertilizer (Wuxal Super, Aglukon, Germany). We measured turgor loss point, root morphological traits (specific root length, root diameter and root tissue density) and root mass ratio under well-watered conditions.

The common garden experiment was carried out at the Ecological Botanical Garden of the University of Bayreuth, Germany (Manuscript 1). 72 plots (1 m x 2 m) were dug, filled with sand and covered with transparent rainout shelters. One individual per species was randomly planted in each plot and in a 20 cm grid with spacing chosen to avoid competition. Plants were initially fertilized and watered for two months to allow for acclimation. 32 of the plots were then exposed to a drought treatment for 10 weeks (3 August – 9 October 2015) by discontinuing irrigation. Soil water potentials in these drought treatment plots declined strongly and reached -1.5 MPa, the permanent wilting point in agricultural plants (Veihmeyer & Hendrickson 1928), within 18 days of ceasing irrigation (Manuscript 1). At the end of the drought treatment, rainout shelters were removed, all plots were irrigated again, and then plants were left under natural conditions until the next spring (for further details on the experiment see Manuscript 1). We measured root allocation (rooting depth, root mass, total root length and again root mass ratio) under irrigated and drought conditions, and their phenotypic plasticity in response to drought. Midday leaf water potentials under drought conditions and whole-plant drought resistance were assessed in the same common garden experiment (Manuscript 1; Manuscript 2). Root mass ratios were significantly correlated in the greenhouse and the common garden experiment (under irrigated conditions; $r = 0.49$, $P < 0.01$), suggesting that any pot effects on root development were limited and that datasets from the greenhouse and the common garden experiment could be usefully combined.

Turgor loss point

Turgor loss point (TLP) was assessed on six healthy individuals for each species based on the osmotic water potential at full turgor (π_o, Manuscript 2). We followed the method in Bartlett *et al.* (2012), for details see Manuscript 2. In short, plants were rehydrated overnight in the dark, and the osmotic water potential of one leaf disk from each individual was measured in a VAPRO 5500 vapor pressure osmometer (Vapor Model 5600, Wescor, Logan, Utah, USA). The osmotic water potential at full turgor was converted to turgor loss point based on the equation $TLP = 0.645 \cdot \pi_o - 0.942$ (Manuscript 2).

Root morphological traits

Root diameter (RDia), specific root length (SRL) and root tissue density (RDen) were assessed on three individuals for each species. Plants in the vegetative growth phase were harvested in March 2016. Roots were washed and the whole root excluding the tap root or rhizome, or a representative subsample of the root (more than half of the whole root system) was scanned at 400 dpi (Epson Perfection V700 Photo Scanner, Japan) and analyzed for root diameter (RDia), root length and root volume with WinRhizo (Regent Instruments Inc., Quebec, Canada). Root samples, the remaining roots and aboveground biomass were separately oven-dried at 70°C for 48 hours. Specific root length (SRL, root length/root mass), root tissue density (RDen, root mass/root volume) and root mass ratio (root mass/plant mass) were calculated.

Root allocation and phenotypic plasticity

Rooting depth, root mass, total root length, root mass ratio, and their phenotypic plasticity were assessed in April 2016. Plants in eight irrigated and eight drought treatment plots were carefully dug out. Rooting depth (RDep) was measured for each individual *in situ* as the depth from the soil surface to the deepest root. Roots and shoots were oven-dried. Root mass (RM), root mass ratio (RMR, root mass/plant mass) and total root length (TRL, root mass*specific root length (see above)) were assessed. Although root mass ratios in the greenhouse and in the common garden experiment were highly correlated (see above), only root mass ratios in the common garden experiment were used in analyses. Phenotypic plasticity of rooting depth (PRDep), root mass (PRM), root mass ratio (PRMR), and total root length (PTRL) were assessed as the respective relative distance plasticity index (RDPI, Valladares, Sanchez-Gomez & Zavala 2006) based on the relative trait difference of all pairs of individuals within a species growing in different conditions (the drought and irrigated treatments), allowing us to test the differences in plasticity among species (RDPI, Valladares, Sanchez-Gomez & Zavala 2006). The

phenotypic plasticity of root total length is the same as for root mass and thus was not analyzed separately.

Root depth distribution

Root depth distribution (RDis) was assessed for 35 species from detailed drawings of whole root profiles (Kutschera 1960; Kutschera, Lichtenegger & Sobotik 1982) for plants growing under natural conditions or on farmlands, where one 'representative' individual was drawn for each species. Root profile figures were scanned and the root fractions along continuous soil profiles were analyzed using ImageJ (Schneider, Rasband & Eliceiri 2012). Root depth distribution (RDis) was calculated by fitting the asymptotic equation (Gale & Grigal 1987): $Y = 1 - RDis^d$ where Y is the cumulative fraction of roots from the soil surface to depth d (cm). High values of RDis indicate a larger proportion of roots in deeper soil layers. We validated that the root depth distribution assessed with this method captured species characteristic root depth distribution patterns, by relating it to the assessment with actual root biomass per soil layer (up to 40 cm) in nine species, using plants growing in monocultures exposed to natural conditions (r = 0.71, P < 0.05; data from Oram *et al.* 2018).

Midday leaf water potentials under drought

Midday leaf water potentials under drought (MWP) were assessed in eight randomly selected drought treatment plots one week after discontinuing irrigation when most plants lost turgor and started wilting (Manuscript 2). Measurements were taken with leaf cutter psychrometers (Merrill Specialty Equipment, Logan, Utah, USA) and a PSYPRO™ water potential system (Wescor, Inc., Logan, Utah, USA) on eight continuous days (August 8th – 15th 2015) with all species in one plot sampled in one day from 11:30h to 12:30h to avoid any bias introduced by sampling date and ensure comparability across species. For details, see Manuscript 2.

Whole-plant drought resistance

Whole-plant drought resistance (DR) was assessed as the response ratio of survival in drought treatment relative to irrigated treatment, i.e. DR = % S_{dry}/% S_{irr}, where % S is alive/initial number in the drought and the irrigated treatment, respectively (Engelbrecht & Kursar 2003; Manuscript 1). Plant survival was assessed based on above-ground organs at the end of the

drought experiment in 2015, corrected for resprouting based on a census in April 2016 (for details see Manuscript 1).

Statistics:

We initially tested if all traits and midday leaf water potentials under drought differed among all species and separately among forbs and grasses with one-way ANOVAs. We then tested if they as well as whole-plant drought resistance differed between forbs and grasses using t-tests. Correlations among traits were assessed with Pearson's correlations and relations of all traits with midday water potentials under drought and whole-plant drought resistance were assessed using univariate regressions. Normality was inspected graphically and tested using Shapiro-Wilk test. Data were log transformed to increase normality when needed (Table 2).

To assess the relative importance of traits for desiccation avoidance and whole-plant drought resistance, we calculated the trait importance values with a random forest algorithm, which specifically evaluates the contribution of each trait to the changes of prediction accuracy of the response when the values of the trait is randomly permuted (Genuer, Poggi & Tuleau-Malot 2010). It is a fully non-parametric statistical method, and therefore avoids the constraints of linear models (Breiman 2001; Genuer, Poggi & Tuleau-Malot 2010; Hapfelmeier & Ulm 2013). Namely, the analysis of the importance value for each trait with linear regressions is problematic if a large number of traits are highly correlated as in our study (Table S2). Removal of traits that are highly correlated with other traits based on variance inflation factor (VIF) values or correlation coefficients (O'Brien 2007; Dormann *et al.* 2013) can overestimate the importance of remaining traits and underestimate the eliminated ones, especially when all traits are included in the experiment due to their hypothesized or known ecological and physiological importance (McDonald 2014).

We additionally used principal components analysis (PCA) to assess trait associations and evaluated trait importance for desiccation avoidance and whole-plant drought resistance by relating species coordinates on principal components axes to species midday leaf water potentials under drought and to whole-plant drought resistance with univariate regressions.

For the prediction purpose, our aim was to provide the most parsimonious models and test the predictive power of *a priori* selected combinations of traits. Multiple regressions were used to select the most parsimonious models based on the Bayesian information criterion (BIC) to

predict drought resistance. Here, we included midday leaf water potentials under drought as a trait to predict species whole-plant drought resistance and removed SRL and total root length in the drought treatment which were highly correlated with other traits (all $|rs| \geq 0.7$, Table S2; Dormann *et al.* 2013). The performance of the final model was evaluated based on explanatory power, i.e. the explained variance of the response variable. The significance of variables in the final models was assessed with multiple regressions by comparing the estimate of the coefficient of each variable with zero using t-test. We quantified the contribution of each individual variable to the explained variance of the best models based on sequential R^2s with weighted averages over orderings which takes care of the dependence of orderings of the traits in the models (Gromping 2006).

To facilitate pragmatic decisions for trait selection to predict species drought resistance, we additionally tested the predictive performances of combinations of 2-3 traits that either had the high explanatory power in the best models (Table 4) or that can be assessed with reasonable experimental and measurement efforts (e.g. allocation under irrigation or root morphological traits).

All analyses were done across all species as well as separately within forbs and grasses to assess differences in the trait importance and in the best predictive models of drought resistance between forbs and grasses.

The variable importance value was assessed using randomForest package (Liaw & Wiener 2002). glmulti package was used for model selection (Calcagno & de Mazancourt 2010). The contribution of each variable in the final models was calculated using relaimpo package with 'pmvd' method (Gromping 2006). The remaining analyses were done using base R packages. All the analyses were done using R (R Core Team 2018).

Results

All traits including turgor loss point as well as midday leaf water potentials under drought varied significantly across all species, as well as within forbs and grasses, respectively (Table 1).

Table 1 The traits, their hypothesized relations to whole-plant drought resistance and desiccation avoidance, i.e. midday leaf water potentials under drought, abbreviations, units and summary statistics.

Traits	Hypotheses	Abbreviation	Unit	(a) all species	(b) species effect	(c) forbs		(d) grasses	(e) life form effect
Performance under drought									
whole-plant drought resistance		DR		0.91 ± 0.02		0.93 ± 0.03		0.90 ± 0.03	n.s.
				(0.46, 1.03)					
midday leaf water potential	+	MWP	MPa	-2.42 ± 0.16	0.62***	-1.86 ± 0.15	>	-2.95 ± 0.23	***
				(-5.68, -1.10)		(0.64***)		(0.50***)	
Water loss control									
turgor loss point	+	TLP	MPa	-1.79 ± 0.03	0.61***	-1.73 ± 0.03	>	-1.85 ± 0.04	*
				(-2.30, -1.49)		(0.68***)		(0.54***)	
Root morphology									
root diameter	-	RDia	mm	0.38 ± 0.06	0.93***	0.42 ± 0.004	>	0.34 ± 0.004	**
				(0.26, 0.59)		(0.71***)		(0.53***)	
specific root length	+	SRL	m/g	173 ± 4.28	0.93***	104 ± 4.33	<	239 ± 10.1	*
				(16.87, 983.96)		(0.68***)		(0.56)	
root tissue density	-	RDen	g/cm^3	0.11 ± 0.02	0.86***	0.14 ± 0.004	>	0.08 ± 0.002	**
				(0.02, 0.27)		(0.64***)		(0.57***)	
Root allocation									
rooting depth dry	+	$RDep_{dry}$	cm	43.77 ± 1.54	0.39***	46.93 ± 2.60	>	40.75 ± 1.48	*
				(29.00, 74.17)		(0.43***)		(0.26***)	
rooting depth irrigated	+	$RDep_{irr}$	cm	33.71 ± 0.78	0.29***	32.96 ± 1.28		34.42 ± 0.93	n.s.
				(20.67, 45.17)		(0.30***)		(0.26***)	
root mass dry	+	RM_{dry}	g	6.25 ± 0.89	0.45***	6.46 ± 1.36		6.04 ± 1.20	n.s.

				(0.57, 25.85)		(0.38***)		(0.58***)	
root mass irrigated	+	RM_{irr}	g	7.32 ± 1.20	0.60***	4.72 ± 0.99	<	9.78 ± 2.02	*
				(1.10, 45.00)		(0.51***)		(0.60***)	
total root length dry	+	TRL_{dry}	m	1783 ± 278	0.73***	1098 ± 70.8		2436 ± 219	n.s.
				(13.15, 21902)		(0.51***)		(0.76***)	
total root length irrigated	+	TRL_{irr}	m	2177 ± 340	0.67***	836 ± 60.0	<	3456 ± 201	*
				(52.99, 14774)		(0.53***)		(0.57***)	
root mass ratio dry	+	RMR_{dry}	g/g	0.66 ± 0.02	0.42***	0.64 ± 0.04		0.68 ± 0.02	n.s.
				(0.37, 0.94)		(0.57***)		(0.19***)	
root mass ratio irrigated	+	RMR_{irr}	g/g	0.69 ± 0.03	0.67***	0.67 ± 0.05		0.70 ± 0.03	n.s.
				(0.21, 0.97)		(0.71***)		(0.58***)	
root depth distribution	+	RDis		0.94 ± 0.01		0.94 ± 0.01		0.94 ± 0.005	n.s.
				(0.86, 0.99)					
Root phenotypic plasticity									
phenotypic plasticity of rooting depth	+	PRDep	cm/cm	0.18 ± 0.01	0.18***	0.21 ± 0.01	>	0.16 ± 0.01	***
				(0.10, 0.32)		(0.19***)		(0.21***)	
phenotypic plasticity of root mass	+	PRM	g/g	0.45 ± 0.02	0.13***	0.45 ± 0.03		0.45 ± 0.02	n.s.
				(0.25, 0.79)		(0.23***)		(0.26***)	
phenotypic plasticity of root mass ratio	+	PRMR	(g/g)/(g/g)	0.17 ± 0.01	0.25***	0.19 ± 0.02		0.14 ± 0.01	n.s.
				(0.06, 0.47)		(0.38***)		(0.27***)	

Given are (a) the mean ± standard error across all 41 species (with minimum and maximum species averages given in parenthesis) and (b) the species effect size (one-way ANOVA) as well as the mean ± standard error within (c) forbs and (d) grasses (with respective species effect size given in parenthesis) and (e) the significance of the differences between forbs and grasses (t-tests). Significance is given as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s. $p > 0.05$. Directions of relations are indicated by + and – as positive and negative relation, respectively. For details on trait measurements and calculations see methods. Whole-plant drought resistance and root depth distribution is only one value per species.

Table 2 Hypothesized and **tested** relations of individual traits with whole-plant drought resistance (DR) and desiccation avoidance, i.e. midday leaf water potentials under drought conditions (MWP).

		all species				forbs				grasses			
Traits	Hypothesis		DR		MWP		DR		MWP		DR		MWP
MWP	(+)	+	0.38 ***			+	0.20*			+	0.58***		
Water loss control													
TLP	(+)	+	0.20**	+	0.36***	+	0.20*	+	0.21*		0.18 n.s.	+	0.34**
Root morphology													
log Rdia	(-)		< 0.01 n.s.		0.04 n.s.	-	0.40**		0.04 n.s.		0.08 n.s.		< 0.01 n.s.
log SRL	(+)		0.03 n.s.	-	0.13*		0.04 n.s.		< 0.01 n.s.		0.16 n.s.		0.03 n.s.
log RD	(-)		0.06 n.s.	+	0.15*		0.09 n.s.		0.07 n.s.		0.02 n.s.		0.03 n.s.
Root allocation													
log RDep $_{dry}$	(+)	+	0.21**	+	0.14*	+	0.21*		0.11 n.s.	+	0.21*		0.05 n.s.
log RDep $_{irr}$	(+)	+	0.15*	+	0.10*		0.07 n.s.		0.07 n.s.	+	0.36**	+	0.54***
log RM $_{dry}$	(+)	+	0.27***	+	0.12*	+	0.40**	+	0.20*	+	0.19*	+	0.19*
log RM $_{irr}$	(+)		0.04 n.s.		< 0.01 n.s.		0.11 n.s.	+	0.31*		0.09 n.s.		0.10 n.s.
log TRL $_{dry}$	(+)		0.05 n.s		< 0.01 n.s.	+	0.28*		0.08 n.s.		< 0.01 n.s.		0.04 n.s.
log TRL $_{irr}$	(+)		< 0.01 n.s.		0.04 n.s.		0.12 n.s.		0.13 n.s.		< 0.01 n.s.		0.01 n.s.
RMR $_{dry}$	(+)	-	0.12*		0.05 n.s.		0.11 n.s.		0.03 n.s.		0.18 n.s.		0.06 n.s.
RMR $_{irr}$	(+)		0.03 n.s.		0.04 n.s.		0.03 n.s.		0.01 n.s.	-	0.35**	-	0.26*
RDis	(+)	+	0.23**	+	0.15*		0.19 n.s.		0.10 n.s.	+	0.42**	+	0.55***
Root phenotypic plasticity													
log PRDep	(+)		0.04 n.s.		0.03 n.s.		0.13 n.s.		< 0.01 n.s.		0.01 n.s.		0.04 n.s.
log PRM	(+)	+	0.13*	+	0.10*		0.18 n.s.		< 0.01 n.s.		0.07 n.s.		0.06 n.s.
log PRMR	(+)		0.01 n.s.		< 0.01 n.s.		0.07 n.s.		0.01 n.s.		0.17 n.s.	+	0.19*

Directions of relations are given as + or - (positive and negative, respectively). R^2 values from univariate regression analyses are given and significance is indicated as * P < 0.05, ** P< 0.01, *** P< 0.001, n.s. P > 0.1. Traits log-transformed to increase normality are indicated. See Table 1 for trait abbreviations.

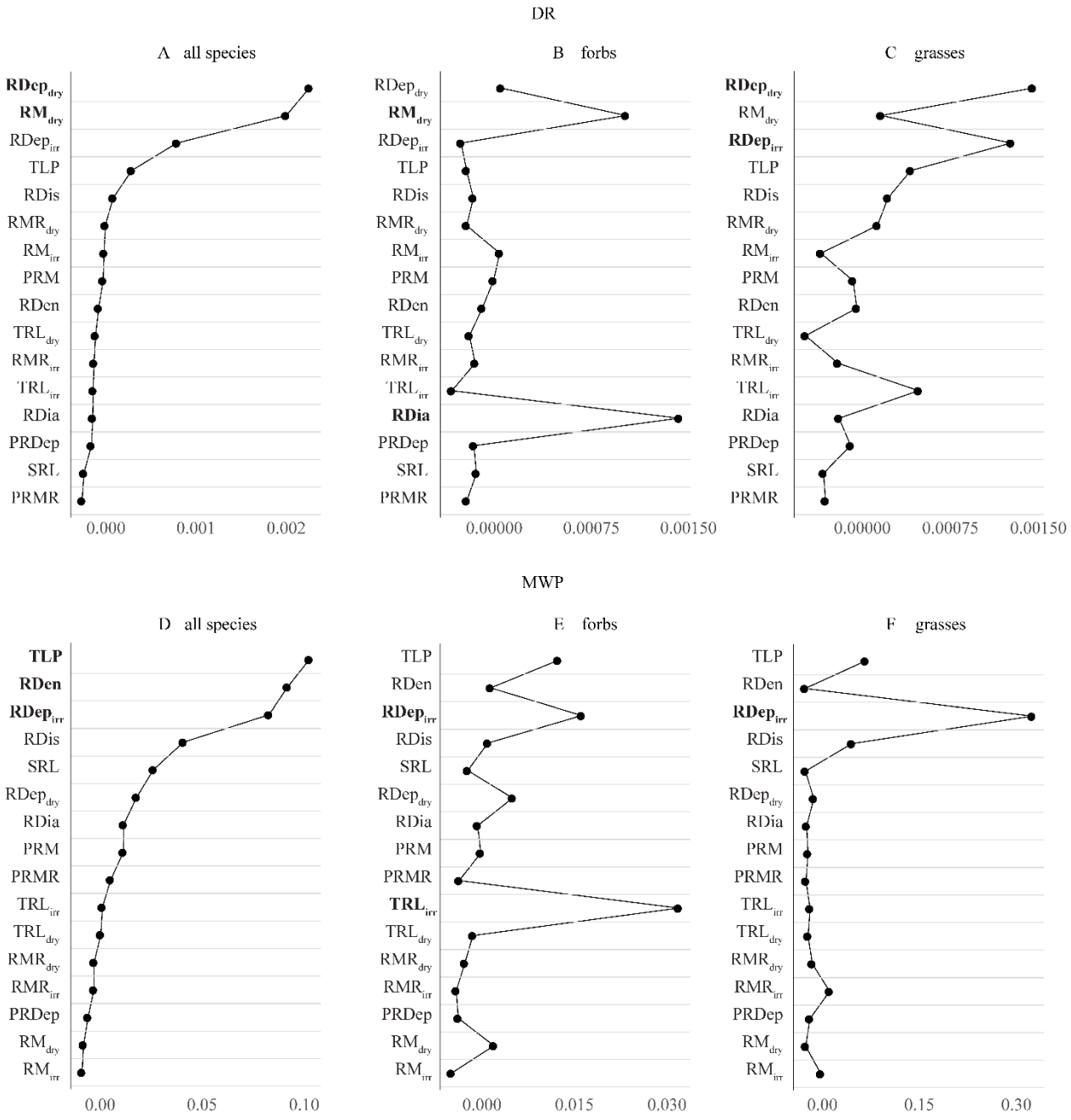

Fig. 1 Differential importance of all 15 root traits and turgor loss point for whole-plant drought resistance (DR; A, B, C) and desiccation avoidance, i.e. midday leaf water potentials under drought (MWP; D, E, F) across all species (A, D) and within forbs (B, E) and within grasses (C, F). Given are variable importance values based on random forest models. Traits are ordered by their importance across all species for whole-plant drought resistance (A) and for desiccation avoidance (D), respectively. The most important traits are highlighted in bold. Note that comparison of trait importance values is useful to compare relative trait contributions within each dataset, but not to evaluate the absolute trait contributions across datasets.

Traits did not vary independently, but several were highly coordinated (Table S2, Fig. S1). Across all species, midday water potentials under drought, indicative of desiccation avoidance, turgor loss point and various root traits were significantly related to whole-plant drought resistance (Table 2). Turgor loss point and root traits were also related to midday water potentials under drought (Table 2). Overall these significant relations of individual traits were consistent with our hypotheses (Table 2). However, some deviations emerged, i.e. for specific root length, root tissue density and root mass ratio, relations were opposite to expected for either drought resistance or midday water potentials under drought (Table 2).

Trait importance for whole-plant drought resistance and desiccation avoidance

Traits differentially contributed to whole-plant drought resistance and desiccation avoidance (i.e. midday leaf water potentials under drought). Consistently, across all species, as well as across forbs and grasses separately, root traits were more important for drought resistance, whereas turgor loss point had relatively minor importance (Fig. 1A, B, C). In contrast, turgor loss point was consistently included among the traits with the highest importance for midday leaf water potentials under drought (Fig. 1D, E, F).

Across all species, for drought resistance, rooting depth in drought treatment was the most important trait following by root mass in the drought treatment (Fig. 1A). For desiccation avoidance, turgor loss point was the most important followed by root tissue density and rooting depth in the irrigated treatment (Fig. 1D). Phenotypic plasticity of root traits in response to drought was not important. The traits with high importance values generally were also the ones that individually showed significant correlations to drought resistance and midday leaf water potentials under drought, while traits with low importance were unrelated to them (Table 2).

Overall, similar results were found in the PCA analysis (Fig. 2). Drought resistance and midday leaf water potentials under drought were both highly correlated with PC2 (r = -0.68 and r = -0.60, respectively, both $p < 0.001$) which had higher positive loadings for rooting depth and root mass in drought treatment (Table 3), but not with any other PCA axes (all $|r| < 0.26$, $P > 0.10$).

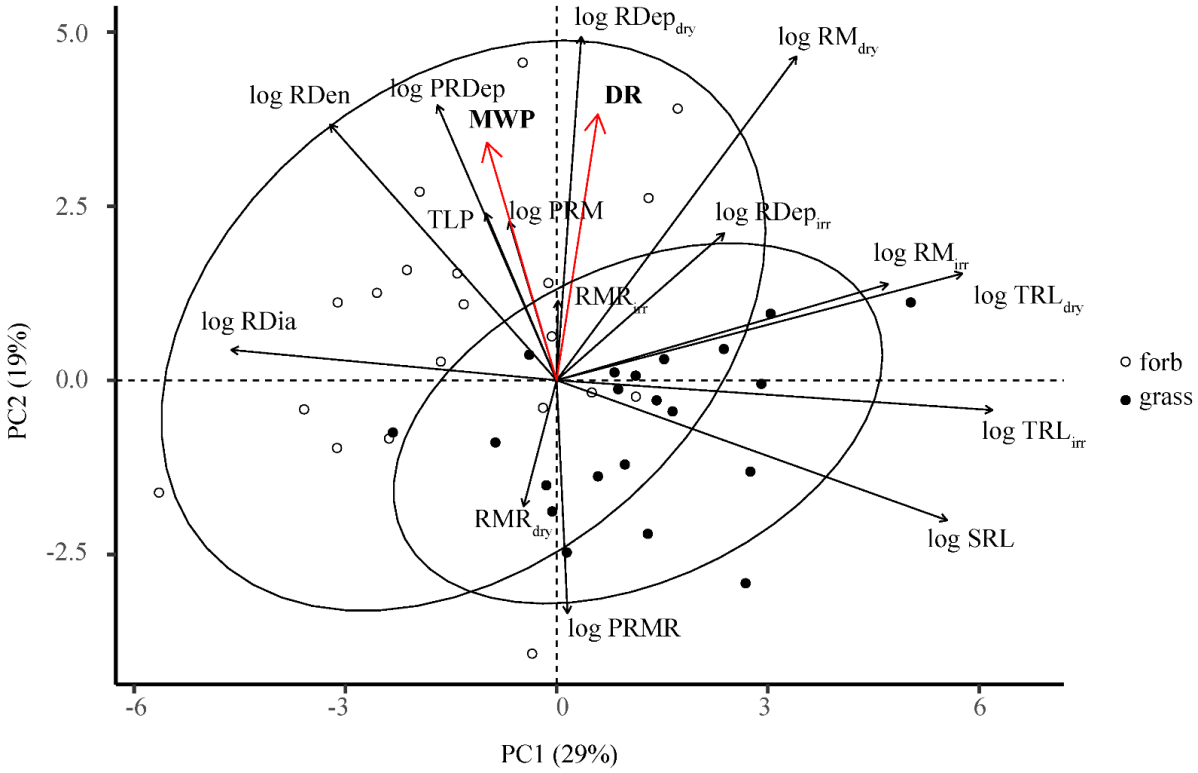

Fig. 2 Results of a principal component analysis of all 15 root traits and turgor loss point. Forbs are given in open and grasses in closed. Circles present the 95% confidence intervals of mean values for forbs and grasses, respectively, on PCA axis 1 (PC1) and axis 2 (PC2). The relative positions of whole-plant drought resistance (DR) and desiccation avoidance, i.e. midday leaf water potentials under drought (MWP) are highlighted with red arrows. For trait abbreviations and trait loadings on PC1 and PC2 see Table 1 and Table 3, respectively.

Differences between forbs and grasses in traits and trait importance for drought resistance

Turgor loss point as well as various but not all root traits pertaining to morphology, allocation and phenotypic plasticity differed between forbs and grasses (Table 1). Specifically, forbs had higher root diameter, root tissue density, rooting depth in drought treatment, phenotypic plasticity of rooting depth and turgor loss point, and lower specific root length and root mass and total root length in irrigated treatment than grasses (Table 1). These differences lead to a separation of the two life forms in the principal component analysis along PC1 and PC2 (Fig. 2). Forbs also maintained higher midday leaf water potentials under drought than grasses (Table 1). Drought resistance did not significantly differ between forbs and grasses (Table 1).

Table 3 Trait loadings on the two main components of the principal component analysis.

	PC1	PC2
logRDep $_{dry}$	0.05	0.77
logRM $_{dry}$	0.54	0.73
logPRDep	-0.27	0.62
logRDen	-0.51	0.58
logPRMR	0.02	-0.53
TLP	-0.16	0.38
logRDep $_{irr}$	-0.11	0.36
RMR_{dry}	0.37	0.33
logPRM	0.87	-0.32
logSRL	-0.07	-0.28
$logRM_{irr}$	0.91	0.24
$logTRL_{dry}$	0.74	0.22
RMR_{irr}	0.00	0.18
logRDia	-0.73	0.07
logTRL $_{irr}$	0.97	-0.07

Traits were sorted by their loadings on PC2, which was highly correlated with both whole-plant drought resistance and desiccation avoidance (midday leaf water potentials under drought). Compare the main text and figure 3.

Traits also differed in their importance for desiccation avoidance and drought resistance between forbs and grasses (Fig. 1 B, C, E, F). Similar to the trend across all species, rooting depth in drought and/or irrigated treatment was the most important for desiccation avoidance and drought resistance in grasses and was among the traits with the high importance for desiccation avoidance but not for drought resistance in forbs. In forbs, total root length in irrigated treatment had the highest importance for desiccation avoidance, and root diameter and root mass in drought treatment were more important than others for drought resistance.

Prediction of whole-plant drought resistance from traits

Across all species, the best model included only two traits, midday leaf water potentials under drought and root mass in drought treatment and could explain 48% of the variance of drought resistance (Table 4). For forbs and grasses, even 84% and 98% variance of drought resistance, respectively, could be explained by the traits in the best models, with substantially different trait combinations in each life forms (Table 4). However, the best models for predicting drought resistance within forbs and grasses selected based on BIC, were inefficient, i.e. six and 13 traits

were included in the final models for forbs and for grasses, respectively. The best models for predicting desiccation avoidance were also analyzed and are presented in Table S3, but not considered further.

To facilitate pragmatic decisions for selecting traits to predict drought resistance of grassland species, we additionally tested the predictive power of combinations of 2-3 traits. These trait sets either consisted of traits with the high explanatory power in the best models (see above, Table 4) or included traits that require limited experimental effort to assess (e.g. allocation under irrigation or root morphological traits). Midday leaf water potentials under drought and root mass in drought treatment, the two traits that were most efficient to predict drought resistance across all species, also performed well in predicting drought resistance within forbs and grasses, explaining 46% and 59% of the variance, respectively (Table 5). Root morphological traits alone predicted drought resistance well within forbs (56%, Table 5), but performed poorly across all species and within grasses. Within grasses, root mass combined with rooting depth in irrigated treatment and root depth distribution explained a large proportion of variance in whole-plant drought resistance (60%, Table 5).

Table 4 The best predictive models for whole-plant drought resistance across all species, within forbs and within grasses based on multiple regressions.

	parameters	estimate	R^2	R^2 scaled
all species			**0.48**	
	intercept	0.97		
	MWP	0.06	0.32***	67%
	log RM dry	0.13	0.16**	33%
forbs			**0.84**	
	intercept	0.81		
	log RDia	-0.72	0.34**	40%
	log RDen	0.30	0.14**	17%
	log PRM	0.18	0.11**	13%
	log RDep dry	0.23	0.11 n.s.	13%
	RMR dry	-0.18	0.08(*)	9%
	RMR irr	-0.24	0.07*	8%
grasses			**0.98**	
	intercept	-16.92		
	MWP	-0.15	0.32(*)	33%
	RMR irr	-3.12	0.14 n.s.	14%
	log RDep irr	2.30	0.10*	10%
	RDis	9.78	0.10*	10%
	log RM dry	0.53	0.08*	8%
	RMR dry	3.28	0.04*	4%
	TLP	-0.98	0.04*	4%
	log RDep dry	0.65	0.04 n.s.	4%
	log PRM	-1.35	0.04*	4%
	log RDen	-0.44	0.03*	3%
	log TRL irr	-0.10	0.02 n.s.	2%
	log PRDep	-1.10	0.02(*)	2%
	log PRMR	-2.75	0.02 n.s.	2%

The respective model parameters and the total variance explained by the model are given in bold. Additionally given are the estimates of the model parameters, variances explained by each variable, its corresponding variable significance, and the relative proportion of model accounted variance explained by each variable scaled to 100% indicated by R^2 scaled. Model selections were based on BIC and the significance of variables in the best models was assessed with multiple regressions by comparing the estimate of the coefficient of each variable with zero using t-test. Note SRL and TRL dry were not included in the predictive model analyses due to their high correlation with other traits. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, (*) $P < 0.1$, n.s. $P > 0.1$.

Table 5 The performances of different combinations of 2-3 traits for predicting whole-plant drought resistance.

		all species			forbs			grasses		
	parameters	estimate	R^2	R^2_{scaled}	estimate	R^2	R^2_{scaled}	estimate	R^2	R^2_{scaled}
best traits across all species			**0.48**			**0.43**			**0.59**	
	intercept	0.99			0.88			1.12		
	MWP	0.06	0.32***	67%	0.03	0.06 n.s.	13%	0.09	0.57***	97%
	log RM $_{dry}$	0.13	0.16**	33%	0.17	0.38*	87%	0.06	0.02 n.s.	3%
best traits across all species,			**0.41**			**0.21**			**0.58**	
	intercept	1.04			1.03			1.15		
with allocation traits	**MWP**	0.07	0.37***	91%	0.06	0.19 n.s.	93%	0.09	0.57***	99%
	log RM $_{irr}$	0.07	0.04 n.s.	9%	0.04	0.02 n.s.	7%	0.03	0.01 n.s.	1%
measured under irrigated conditions			**0.42**			**0.23**			**0.58**	
	intercept	0.62			0.69			0.85		
	MWP	0.07	0.36***	86%	0.07	0.19 n.s.	85%	0.09	0.57***	98%
	log RM $_{irr}$	0.04	0.02 n.s.	4%	-0.002	< 0.01 n.s.	0%	0.03	< 0.01 n.s.	1%
	log RDep $_{irr}$	0.28	0.04 n.s.	10%	0.25	0.03 n.s.	15%	0.18	0.01 n.s.	1%
root morphology traits			**0.07**			**0.56**			**0.24**	
	intercept	1.01			0.64			1.37		
	log RDia	-0.05	< 0.01 n.s.	3%	-0.59	0.34 n.s.	62%	-1.02	0.05 n.s.	21%
	log SRL	0.004	< 0.01 n.s.	0%	0.16	0.07 n.s.	13%	-0.68	0.15 n.s.	62%
	log RDen	0.12	0.06 n.s.	97%	0.27	0.14*	25%	-0.51	0.04 n.s.	18%
allocation traits measured			**0.33**			**0.27**			**0.60**	
	intercept	1.29			-0.20			-2.84		
under irrigated conditions	**log RM $_{irr}$**	0.01	< 0.01 n.s.	0%	0.08	0.09 n.s.	34%	0.08	0.03 n.s.	5%
	log RDep $_{irr}$	0.56	0.14 n.s.	42%	0.10	0.01 n.s.	3%	0.98	0.38 n.s.	63%
	RDis	1.42	0.19*	57%	0.99	0.17 n.s.	64%	2.28	0.19 n.s.	32%
turgor loss point and allocation traits			**0.29**			**0.26**			**0.43**	
	intercept	0.64			1.36			-0.61		
measured under irrigated conditions	**TLP**	0.26	0.18**	61%	0.29	0.19 n.s.	71%	0.17	0.08 n.s.	19%
	log RM $_{irr}$	0.03	0.01 n.s.	2%	0.09	0.07 n.s.	29%	0.04	0.01 n.s.	2%
	log RDep $_{irr}$	0.48	0.11 n.s.	36%	0.02	< 0.01 n.s.	0%	1.16	0.33*	78%

Traits combinations were selected based on their high explanatory power in the best models (Table 4) or their relative ease of measurement. See Table 4 for the details of table contents.

Discussion

Importance of traits for whole-plant drought resistance and desiccation avoidance

To our knowledge, this is the first study that directly evaluated the relative importance of both turgor loss point and root traits for comparative whole-plant drought resistance across multiple grassland species. Across all species as well as within forbs and grasses, various root traits, such as high rooting depth and root mass, had the highest importance for both midday leaf water potentials under drought (i.e. desiccation avoidance) and whole-plant drought resistance, while turgor loss point had only high importance for midday leaf water potentials under drought (Fig. 1). These results suggested that root traits that maximize water uptake were more important for grassland species to resist drought than turgor loss point decreasing water loss at leaf level. The assessments of the relative importance of turgor loss point and root traits for drought resistance are rare. Consistent with our results, Buckland *et al.* (1997) found that high rooting depth enabled species to maintain high water content and high abundance under drought while leaf keeping turgid, supporting the high importance of root traits for promoting drought resistance in grassland species. Minimizing water loss at leaf level through high turgor loss point (i.e. early loss of leaf turgor) has trade-offs with carbon gain by inducing stomatal closure and can lead to carbon-starvation induced drought mortality (McDowell *et al.* 2008). Further, loss of turgor impedes turgor pressure dependent processes that are associated with growth, including cell formation, expansion and metabolism (Boyer 1968; Hsiao *et al.* 1976). Maximizing water uptake through root traits can allow leaf to maintain turgor and photosynthesis under drought, and therefore free plants from these constraints. Although this may lead to a trade-off in biomass allocation between root and leaf, low root diameter and root tissue density can weaken this trade-off by enabling plants to grow longer roots with less biomass (i.e. high specific root length; Comas *et al.* 2013; Ma *et al.* 2018). Other leaf traits, such as low specific leaf area, low leaf cuticle conductance and high leaf water potentials under stomatal closure are also important mechanisms that reduce water loss through leaves (Choat *et al.* 2018). For example, low specific leaf area was significantly related to high midday leaf water potentials under drought (Perez-Ramos *et al.* 2013), confirming the importance of minimizing water loss at leaf level for desiccation avoidance. High water potentials at stomatal closure (i.e. more efficient at water loss control) enabled plants to maintain high productivity under drought (Konings, Williams & Gentine 2017). However, the importance of these mechanisms at leaf level in promoting drought resistance may be limited in grassland species.

Many of grassland species showed rapid leaf death under drought and can resprout without any living aboveground biomass when soil water stress alleviated (Volaire, Thomas & Lelievre 1998; Manuscript 1). Consistent with our results (but on much fewer species), Perez-Ramos *et al.* (2013) and Zwicke *et al.* (2015) found that root traits that maximize water uptake such as rooting depth were significantly related to whole-plant drought resistance while leaf traits that minimize water loss such as leaf area and specific leaf area were unrelated to it. These results collectively highlighted the importance of root traits in promoting drought resistance in grassland species.

In grasslands, half of the root biomass is located in the top 10 cm of soil and 80-90% are in top 30 cm (Garwood & Sinclair 1979; Jackson *et al.* 1996; Ma *et al.* 2008). Water in the surface soil layers is quickly exhausted during drought (De Kauwe *et al.* 2015), suggesting that root traits that increase species access to deep soil water which remains available longer under drought may have higher importance for maximizing water uptake under drought and for promoting drought resistance. We found that rooting depth and root mass had the highest importance for whole-plant drought resistance across all species as well as in forbs and grasses (Fig. 1A, B, C). Consistently, previous studies found that high rooting depth increased the amount of available soil water (Barkaoui, Roumet & Volaire 2016), allowed plants to maintain high leaf water potentials under drought (Guderle *et al.* 2018), and promoted species drought survival at individual (Volaire 2008; Perez-Ramos *et al.* 2013; Zwicke *et al.* 2015) and community levels (Barkaoui, Roumet & Volaire 2016), supporting that a root system allowing plants to access deeper soil layers is of paramount importance for high drought resistance in grassland species.

Root morphological traits also contributed to desiccation avoidance and drought resistance, such as root tissue density and root diameter (Fig. 1). The positive effect of root tissue density on midday leaf water potentials under drought was opposite to our expectation that low root tissue density enables plants to build up long root per unit biomass and thus to maintain high leaf water potentials under drought. Alternatively, high root tissue density can reflect a high number of small and highly lignified xylem vessels that lead to high resistance to embolism and permit efficient and continuous water transport in roots even under drought (Wahl & Ryser 2000). This may underly the significant correlation between root tissue density and midday leaf water potentials under drought we observed. The highest importance of low root diameter for promoting whole-plant drought resistance was only found in forbs. Forbs generally have tap

roots (Weaver 1958) which confer them the ability to penetrate hard and dry soils and to reach deep soils layers (Materechera *et al.* 1992) but accounting a large part of root mass (Weaver 1958) and serving little functions in water uptake (Freschet & Roumet 2017). Low root diameter in forbs can enable plants to build more fine roots per biomass allocated to increase root surface area for the soil exploration and water uptake (Ma *et al.* 2018). Grasses with thin fibrous roots may less benefit from it as suggested by our result that root diameter had low importance for whole-plant drought resistance in grasses (Fig. 1C).

High phenotypic plasticity of root structure and allocation in response to drought can allow plants to gain access to deep soil layers under water stress (Hoeppner & Dukes 2012; Larson & Funk 2016). However, the significant correlations between root traits under irrigated and drought conditions suggested that species water uptake capacity was largely determined by inherent root characteristics and the importance of the plasticity of root traits may be minor. Indeed, an important role of phenotypic plasticity of root traits for drought resistance and desiccation avoidance was not supported in our study (Fig. 1). This is contrast to results from previous studies that high phenotypic plasticity of root length and rooting depth enabled plants to inhabit dry habitats (Ma *et al.* 2008) or to maintain high productivity under drought (Hoekstra *et al.* 2014), while others showed that responses of root traits to drought were species-specific and no consistent patterns emerged (Larson & Funk 2016). Further studies that directly relate phenotypic plasticity of traits to comparative whole-plant drought resistance are needed to fully understand the role of phenotypic plasticity of traits for drought resistance.

Differences between forbs and grasses

Forbs and grasses have been treated as two functional groups to simplify and explore ecological questions such as changes in ecosystem services under land use and climate change (Díaz & Cabido 2001), due to their detected broad differences in root and leaf traits (Weaver 1968; Craine *et al.* 2001; Reich *et al.* 2003; Nippert & Knapp 2007; Tucker, Craine & Nippert 2011). However, if and how these differences can lead to a difference in performances under drought between two functional groups are still unclear. Here we found that they differed in root traits and turgor loss point, allowing forbs to maintain higher midday leaf water potentials under drought (Table 1). This aligns with previous studies documenting that higher rooting depth, root tissue density and leaf water potentials at stomatal closure in forbs than grasses (Weaver 1968; Tucker, Craine & Nippert 2011). Despite these group differences, large variations existed within each group and explained by species identity, resulting in the similar whole-plant

drought resistance between two groups (Table 1). Different species compositions used in previous studies thus could explain why forbs were found to have higher (Tilman & El Haddi 1992; Skinner & Comas 2010), lower (Hoover, Knapp & Smith 2014), and similar drought resistance than grasses (Fay *et al.* 2002). Nevertheless, the differences between forbs and grasses support the functional and ecological relevance of categorizing species based on their life forms and may provide explanations to the shifts in grassland functional composition and ecosystem services under drought (Hoover, Knapp & Smith 2014).

Predictions of species drought resistance from trait values

The comparative assessments of species drought resistance are experimental demanding (Manuscript 1) or require spatially extensive sampling and long-term monitoring species performances under nature drought (Shipley *et al.* 2017). A trait-based method can be used to predict species drought resistance and forecast community dynamics under drought (McGill *et al.* 2006; Shipley *et al.* 2016). Several studies have tested associations of functional traits, such as physiological traits governing leaf gas exchange or morphological traits of leaves and roots, with species distributions along soil water gradients (Tucker, Craine & Nippert 2011; Craine *et al.* 2012; Ocheltree, Nippert & Prasad 2016; Belluau & Shipley 2017; Shipley *et al.* 2017; Belluau & Shipley 2018). However, their models are difficult to be translated for predicting species capacity to survive drought due to the weak relation between drought resistance and distribution (see introduction). By directly relating key traits to whole-plant drought resistance across multiple species, we found that only two traits, midday leaf water potentials under drought and root mass under drought, can provide high predictive power for whole-plant drought resistance across all grassland species, as well as within forbs and within grasses (Table 5). To comparatively assess them, however, an extensive experimental effort is required. Traits under irrigated conditions are relatively easy to assess and can be used as proxies of trait values under drought conditions (Table S2). Combinations of these traits did not consistently explain high variance of whole-plant drought resistance across all species, as well as within forbs and within grasses. However, when forbs and grasses were treated separately, the combination of root morphological traits provided high predictive power for forbs, and of root mass and rooting depth under irrigated conditions and root depth distribution for grasses (Table 5). The highest importance of root morphological traits for whole-plant drought resistance in forbs and root allocation traits in grasses support the use of different predictive models for each group (Fig. 1B, C). Admittedly, these models predict the survival of individual species under drought

conditions, without competition and/or facilitation among species. However, previous studies showed that species performances under drought were species-specific, suggesting that the role of biotic interactions may be minor under drought (Pfisterer & Schmid 2002). Nevertheless, the next step will be to assess the predictive power of our models for the observed changes of grasslands that were induced by drought at community level, such as in Biodiversity Exploratories (Fischer *et al.* 2010).

Conclusions

Our study highlighted the importance of root traits, especially the ones increasing species access to more reliable water in deep soils, for maintaining high leaf water potentials under drought and promoting drought resistance. The inclusion of key root traits with the higher importance and removal of uninformative traits conferred us the parsimonious models with high predictive power for species differential drought resistance. These results provided a practical guide for trait selection schemes. The differentiated drought resistance and traits within forbs as well as within grasses may lead to big changes of species and functional compositions in grasslands under climate change.

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Author contributions

B.M.J.E. and S.S. designed the research, S.S., E.J. performed the experiments, S.S. and B.M.J.E. analyzed the data, S.S., E.J. and B.M.J.E. wrote the manuscript.

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Supporting information

Table S1 List of the 41 temperate grassland species in our study with family and life form.

Species	Family	Life form
Achillea millefolium L.	Asteraceae	Forb
Agrostis capillaris L.	Poaceae	Grass
Agrostis stolonifera L.	Poaceae	Grass
Alopecurus pratensis L.	Poaceae	Grass
Anthoxanthum odoratum L.	Poaceae	Grass
Arrhenatherum elatius (L.) P.Beauv. ex J.Presl & C.Presl.	Poaceae	Grass
Brachypodium pinnatum (L.) P.Beauv.	Poaceae	Grass
Briza media L.	Poaceae	Grass
Bromus hordeaceus L.	Poaceae	Grass
Centaurea jacea L.	Asteraceae	Forb
Cerastium holosteoides Fr.	Caryophyllaceae	Forb
Cirsium oleraceum (L.) Scop.	Asteraceae	Forb
Crepis biennis Lapeyr.	Asteraceae	Forb
Dactylis glomerata L.	Poaceae	Grass
Daucus carota L.	Apiaceae	Forb
Elymus repens (L.) Gould	Poaceae	Grass
Festuca guestfalica Boenn. ex Rchb.	Poaceae	Grass
Festuca ovina L.	Poaceae	Grass
Festuca pratensis Huds.	Poaceae	Grass
Festuca rubra L.	Poaceae	Grass
Galium mollugo L.	Rubiaceae	Forb
Helictotrichon pubescens (Huds.) Schult. & Schult.f.	Poaceae	Grass
Holcus lanatus L.	Poaceae	Grass
Lathyrus pratensis L.	Fabaceae	Forb
Leontodon autumnalis L.	Asteraceae	Forb
Leucanthemum vulgare (Vaill.) Lam.	Asteraceae	Forb
Lolium perenne L.	Poaceae	Grass
Lotus corniculatus L.	Fabaceae	Forb
Phleum pretense L.	Poaceae	Grass
Plantago lanceolata L.	Plantaginaceae	Forb
Poa pratensis L.	Poaceae	Grass
Poa trivialis L.	Poaceae	Grass
Prunella vulgaris L.	Lamiaceae	Forb
Ranunculus acris L.	Ranunculaceae	Forb
Ranunculus bulbosus L.	Ranunculaceae	Forb
Ranunculus repens L.	Ranunculaceae	Forb
Rumex acetosa L.	Polygonaceae	Forb
Rumex crispus L.	Polygonaceae	Forb
Taraxacum officinale (L.) Weber ex F.H.Wigg.	Asteraceae	Forb
Trisetum flavescents (L.) P. Beauv.	Poaceae	Grass
Vicia cracca L.	Fabaceae	Forb

Table S2 Correlations among midday leaf water potentials under drought, turgor loss point and root traits across 31 species.

	MWP	TLP	log RDia	log SRL	log RDen	log $RDep_{dry}$	log $RDep_{irr}$	log RM_{dry}	log RM_{irr}	log TRL_{dry}	log TRL_{irr}	RMR_{dry}	RMR_{irr}	RDis	log PRDep	log PRM	log PRMR
MWP	1.00																
TLP	0.60***	1.00															
log RDia	0.20	0.08	1.00														
log SRL	-0.36*	-0.27	-0.69***	1.00													
log RDen	0.39*	0.34*	0.17	-0.75***	1.00												
log $RDep_{dry}$	0.37*	0.17	0.22	-0.17	0.22	1.00											
log $RDep_{irr}$	0.32*	0.22	-0.14	0.08	0.08	0.48**	1.00										
log RM_{dry}	0.35*	0.18	-0.29	0.23	0.13	0.48**	0.29	1.00									
log RM_{irr}	0.03	-0.01	-0.40*	0.38*	-0.04	0.22	0.51***	0.49**	1.00								
log TRL_{dry}	-0.02	-0.07	-0.63***	0.80***	-0.42**	0.18	0.23	0.77***	0.55***	1.00							
log TRL_{irr}	-0.20	-0.18	-0.66***	0.84***	-0.49**	0.02	0.35*	0.43**	0.82***	0.82***	1.00						
RMR_{dry}	-0.23	-0.06	-0.06	-0.01	0.11	-0.32*	-0.08	-0.15	-0.07	-0.10	-0.05	1.00					
RMR_{irr}	-0.21	-0.10	-0.31*	-0.08	0.42**	-0.19	-0.16	0.18	0.07	0.06	-0.01	0.46**	1.00				
RDis	0.38*	0.20	< 0.01	-0.12	0.33	0.40*	0.29	0.30	0.17	0.11	0.03	-0.37*	0.03	1.00			
log PRDep	0.18	-0.02	0.22	-0.26	0.35*	0.52***	-0.19	0.25	-0.19	-0.02	-0.27	-0.21	0.11	0.39*	1.00		
log PRM	0.32*	0.21	0.10	-0.01	-0.02	0.26	-0.04	0.40**	-0.48**	0.24	-0.29	-0.07	-0.05	0.18	0.27	1.00	
log PRMR	-0.05	-0.02	0.14	0.18	-0.41**	-0.17	0.15	-0.35*	-0.10	-0.09	0.05	0.14	-0.46**	0.04	-0.34*	0.12	1.00

Pearson's correlation coefficients and corresponding significances are given, with * P values < 0.05, ** P values < 0.01, *** P values < 0.001.

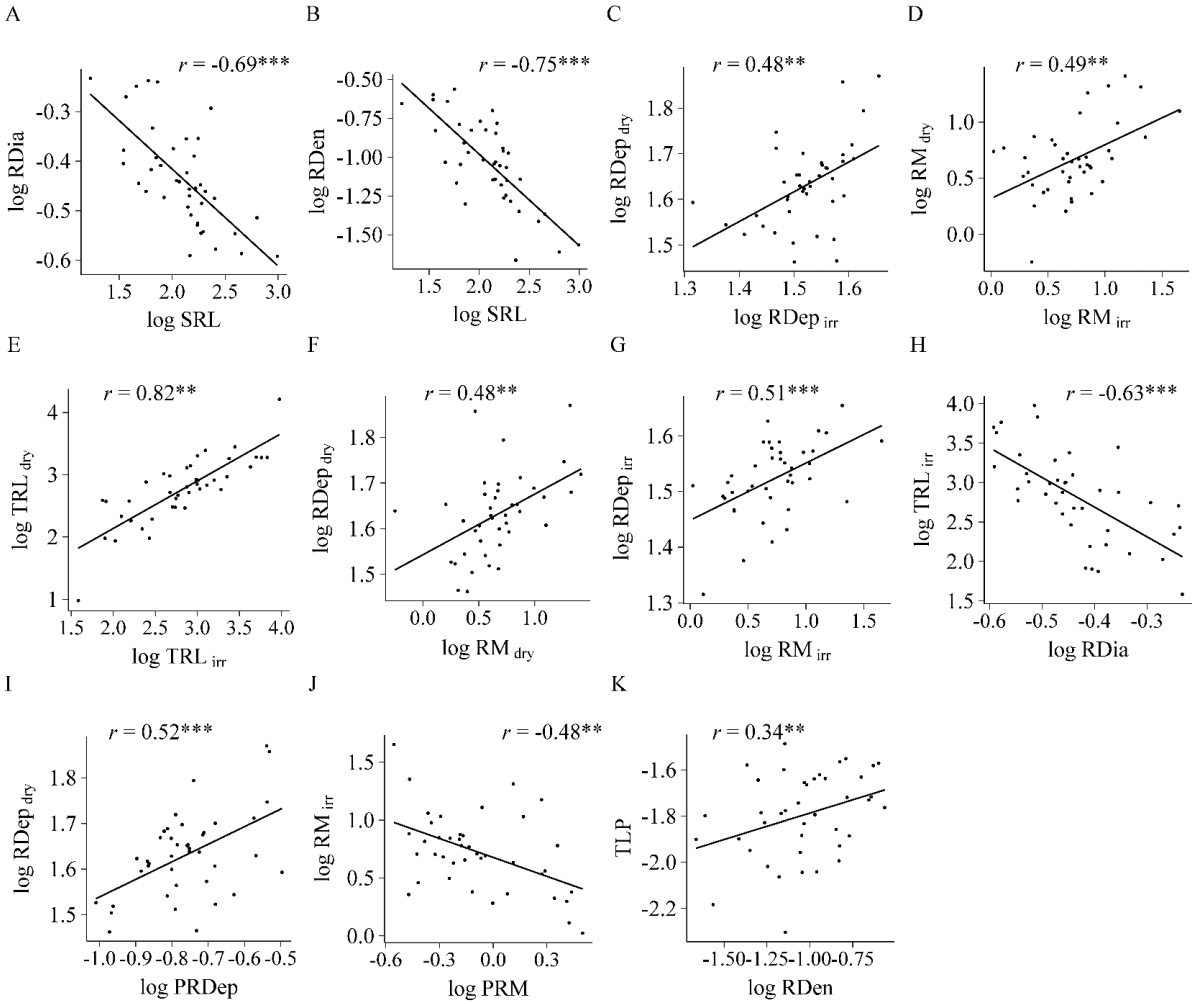

Fig. S1 Examples of correlations between traits, i.e. between root morphology (A)-(B), between root allocation (C)-(G), between root morphology, allocation and phenotypic plasticity (H)-(J), and between root traits and turgor loss point (K). Given are Pearson's correlation coefficients and corresponding significance. See Table 1 for trait abbreviations and Table S2 for the full list of correlations among traits. *** $P < 0.001$, ** $P < 0.01$.

Table S3 The best multivariable regression models for desiccation avoidance, i.e. midday leaf water potentials under drought across all species, within forbs and within grasses based on multiple regressions.

	parameters	estimate	R^2	R^2 scaled
all species			**0.54**	
	intercept	-2.45		
	TLP	2.63	0.32***	59%
	log RDia	3.29	0.09*	16%
	RDis	6.01	0.07 n.s.	13%
	log RM $_{dry}$	0.72	0.06(*)	12%
forbs			**0.83**	
	intercept	-14.67		
	log RDep $_{dry}$	10.01	0.14**	16%
	log RDia	-8.05	0.12*	15%
	log RM $_{dry}$	1.89	0.10*	12%
	log PRDep	-5.58	0.10**	12%
	log RDep $_{irr}$	-9.94	0.09**	10%
	RDis	5.53	0.09 n.s.	10%
	log TRL $_{irr}$	-0.79	0.07(*)	8%
	log PRM	-2.18	0.06*	8%
	log PRMR	3.46	0.06*	8%
			0.98	
	intercept	-63.65		
	log RDep $_{irr}$	8.58	0.72**	74%
	RDis	36.78	0.12**	12%
	TLP	-2.39	0.04(*)	4%
	log PRM	-2.27	0.02**	3%
	log PRMR	16.55	0.02*	2%
	log RDia	3.98	0.02*	2%
	log PRDep	-4.38	0.01*	1%
	log TRL $_{irr}$	1.21	0.01*	1%
	log RM $_{dry}$	-0.80	0.01n.s.	1%
	log RDep $_{dry}$	2.06	0.00n.s.	0%

The respective model parameters and the total variance explained by the model are given in bold. Additionally given are the estimates of the model parameters, variances explained by each variable, its corresponding variable significance, and the relative proportion of model accounted variance explained by each variable scaled to 100% indicated by R^2 $_{scaled.}$ Model selections were based on BIC and the significance of variables in the best models was assessed with multiple regressions by comparing the estimate of the coefficient of each variable with zero using t-test. Note SRL and TRL $_{dry}$ were not included in the predictive model analyses due to their high correlation with other traits. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, (*) $P < 0.1$, n.s. $P > 0.1$.

Manuscript 4: Trait coordination and importance for drought performance in temperate grassland species

Trait coordination and importance for drought performance in temperate grassland species

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Abstract

Drought intensity and frequency are forecasted to increase under climate change. This will strengthen the effects of drought on grasslands. Understanding the traits underlying differential species drought resistance are urgently needed. Numerous traits are involved in drought resistance with their importance likely to differ. Yet, assessments including a comprehensive set of traits, their coordination and relative importance for drought resistance are lacking in grassland species. This largely impedes our understanding of mechanisms of drought resistance and the prediction of effects of drought on grasslands. In this study, we assessed 38 physiological, morphological and anatomical traits that are known or hypothesized to determine drought resistance in 41 temperate grassland species (20 forbs and 21 grasses). We assessed the relations among them and evaluated their importance for differential species drought resistance by linking them to four drought performance parameters, i.e. whole-plant drought resistance of both survival and growth, midday leaf water potential under moderate drought and species association to habitat dryness. We found that many traits were coordinated, traded-off or were independent, and no main axes of trait variation emerged. Further, trait importance and their ranks differed within and across each drought performance parameter. Forbs and grasses had overall similar drought resistance but differed in their traits and in the ranks of trait importance for each drought performance parameter. Collectively, our results suggest that many trait combinations exist and lead to the whole range of drought resistance. It is difficult to generalize traits underlying drought resistance from one drought parameter or functional group to others. Nevertheless, our study can provide practical guides for trait selection by highlighting few traits of high importance for each drought performance parameter.

Introduction

Grasslands cover one-third of the land surface, have high biodiversity and provide important ecosystem services (Scurlock & Hall, 1998; Gibson, 2009). They experience periods of drought (Tilman & El Haddi, 1992; Grime *et al.*, 2008; Gibson, 2009). Drought resistance differs among grassland species (Perez-Ramos *et al.*, 2013; Zwicke *et al.*, 2015; Manuscript 1). This leads to species loss under drought, and influences species abundance and distribution and grassland composition (Weaver, 1968; Tilman & El Haddi, 1992; Buckland *et al.*, 1997; Grime *et al.*, 2008). Under climate change scenario, drought frequency and intensity are predicted to increase (IPCC, 2014). This is likely to strengthen the impacts of drought on grasslands. Therefore, understandings of the traits underlying differential species drought resistance, their coordination and importance are urgently needed.

Various traits have been hypothesized to be important for drought resistance (Table 1). The importance of these traits for grassland species have been tested in many studies, such as root traits (Tucker *et al.*, 2011; Zwicke *et al.*, 2015), gas exchange traits (Tucker *et al.*, 2011; Belluau & Shipley, 2018), or leaf water potentials at stomatal closure (Tucker *et al.*, 2011; Craine *et al.*, 2013) and turgor loss point (Ocheltree *et al.*, 2016; Manuscript 2) or specific leaf area (SLA; Zwicke *et al.*, 2015; Shipley *et al.*, 2017; Belluau & Shipley, 2018). However, a conclusion about traits underlying drought resistance is difficult because different sets of traits were used in each study. Drought resistance may involve multiple traits pertaining to many processes, such as water uptake, water transport, water storage, water loss, membrane vulnerability and carbon gain, with their importance likely to differ (Choat *et al.*, 2018). Lacking evaluation of the relative importance of traits pertaining to the same and different processes impedes our understanding of mechanisms of species drought resistance and the prediction of it (but see Medeiros *et al.*, 2018 for woody species). Further, trait relations are complicated, and they may coordinate or have trade-offs with other traits, or independently to influence drought resistance. For example, maximizing water uptake through root traits may reduce the aboveground biomass allocation to increase carbon gain and membrane stability under drought, while low root diameter alone or with low root density can weaken this trade-off by enabling high root length per biomass allocated, i.e. high specific root length (Ma *et al.*, 2018). Stomatal closure and species resistance to xylem embolism are suggested to tightly coordinated to maximize carbon gain under drought, however, this coordination was only found in a limited number of species, and with increasing resistance to embolism their

coordination gets looser (Martin-StPaul *et al.*, 2017). Yet, trait covariations may be captured by main axes, such as leaf economics spectrum (LES) simplifying trait relations by categorizing them as 'fast' or 'slow' growth traits (Wright *et al.*, 2004). Reich (2014) suggested that the 'fast to slow' economics spectrum may exist at whole-plant level across leaves, stems and roots, and be related to drought resistance by integrating important traits that are associated with water uptake, water storage, water transport, water loss and carbon gain. This spectrum, if existed, would greatly reduce the complexity of trait coordination and facilitate the prediction of the consequences of drought.

Previous studies have used different drought performance parameters to assess the associations between traits and drought resistance with the assumption that the trait importance for drought resistance should be consistent regardless of the parameter that is used, such as whole-plant drought resistance of survival (Volaire, 2008; Perez-Ramos *et al.*, 2013; Zwicke *et al.*, 2015), whole-plant drought resistance of growth (Weißhuhn *et al.*, 2011; Perez-Ramos *et al.*, 2013; Zwicke *et al.*, 2015), and species association to habitat dryness (Cingolani *et al.*, 2007; Tucker *et al.*, 2011; Craine *et al.*, 2013; Marteinsdottir & Eriksson, 2014; Lens *et al.*, 2016; Ocheltree *et al.*, 2016; Shipley *et al.*, 2017; Belluau & Shipley, 2018). However, the relations between these parameters are actually complex and weak in grassland species. For example, both positive and negative relations between whole-plant drought resistance of survival and growth were found in previous studies (Volaire *et al.*, 1998; Volaire & Norton, 2006; Rivero *et al.*, 2007; Perez-Ramos *et al.*, 2013; Zwicke *et al.*, 2015), and whole-plant drought resistance of both survival and growth were weakly related to species distribution along moisture gradients (Manuscript 1). These results suggest that maybe different trait or set of traits influence different drought performance parameters. Further, the maintenance of plant water status under drought, i.e. midday leaf water potentials under drought, is another important drought performance parameter. It reflects plants efficiency of avoiding desiccation by integrating several key plant structural and physiological traits such as rooting depth, leaf water potentials at turgor loss point and at stomatal closure. It also determines the probability of hydraulic dysfunction and thus plants mortality (Choat *et al.*, 2018). Traits that are important for maintenance of high midday leaf water potentials under drought were found to contribute to high whole-plant drought resistance of survival (Perez-Ramos *et al.*, 2013; Zwicke *et al.*, 2015; Manuscript 2), supporting that differences in maintenance of high midday leaf water potentials under drought are important in driving the differential drought resistance in grassland plants. However, if the trait importance for midday leaf water potentials under drought and whole-

plant drought resistance of survival still holds for whole-plant drought resistance of growth and species association to habitat dryness is unknown. An evaluation of trait importance for all these drought performance parameters can help us to better understand the traits underlying drought resistance.

Forbs and grasses have been used as two functional groups under the assumptions that they have certain key traits that are different (Skarpe, 1996) and thus show differential responses to environmental conditions (Diaz & Cabido, 1997). Regarding to drought resistance, many traits were found to significantly differ between these two groups. For instance, forbs were found to lose turgor (Manuscript 2) and close stomata at higher leaf water potentials (Tucker *et al.*, 2011), had deeper root systems (Weaver, 1968), but lower biomass fraction allocated to root (Reich *et al.*, 2003) and higher root diameter (Craine *et al.*, 2001) than grasses. If these trait differences can result in differences in drought performance between two groups not only depends on the magnitude of the differences but also on the importance of traits that are different. Trait importance for drought performance may differ between forbs and grasses as suggested by findings that forbs reduced stomatal conductance under drought (Fay *et al.*, 2002), while grasses allocated more biomass to deep roots (Skinner & Comas, 2010). The direct assessment of trait importance for each group allows to test if trait importance differs between forbs and grasses.

In this study we addressed the following questions: (1) how are individual traits relevant for plant drought resistance coordinated and what are the trade-offs; (2) what is the importance of individual traits for various drought performance parameters; (3) do traits and their importance differ between forbs and grasses.

We measured a comprehensive set of 38 physiological, morphological and anatomical traits pertaining to water uptake, water transport, water storage, water loss, membrane vulnerability and carbon gain in 41 common temperate grassland species including both forbs (20 species) and grasses (21 species). All traits were known or hypothesized to influence drought resistance (Table 1). We also assessed four drought performance parameters, i.e. whole-plant drought resistance of survival, whole-plant drought resistance of growth, midday leaf water potential under moderate drought and species association to habitat dryness (Table 1).

Materials and Materials

Plant material

41 temperate grassland species (20 forbs and 21 grasses) common in Germany were included in our study (Table S1). Species were selected based on the following criteria: (1) high abundance and frequency in grassland plots in Biodiversity Exploratories in three areas across Germany (Schorfheide-Chorin, Hainich-Dün and Schwäbische Alb; Schorfheide-Chorin, Hainich-Dün and Schwäbische Alb; Fischer *et al.*, 2010), (2) association with a wide range of soil moisture availability (Ellenberg indicator values for soil moisture from 3 to 7, Ellenberg indicator values for soil moisture from 3 to 7, Ellenberg *et al.*, 1991), and (3) representing the dominant life history strategy and photosynthetic pathway in European temperate grasslands, perennial and C3 (Ellenberg *et al.*, 1991; Collatz *et al.*, 1998). Seeds were bought from commercial seed suppliers (Rieger-Hofmann GmbH and Saaten Zeller, Germany) and germinated in a greenhouse in mid-March 2015. Plants were transplanted to pots in a greenhouse and to a common garden experiment. The same soil substrate (97% sand, 2% silt and 1% clay) was used in the greenhouse and common garden.

Growing conditions

In the greenhouse, plants were growing in pots (7 cm diameter × 35.6 cm depth), watered twice per day and frequently fertilized with a full fertilizer solution (Wuxal Super, Aglukon, Germany).

The common garden drought experiment was carried out at the Ecological Botanical Garden of University of Bayreuth, Germany. One individual per species was randomly transplanted into 72 field experimental plots (1 m x 2 m) under transparent rainout shelters in the first week of June 2015. Plants were planted in a 20 cm grid in each plot to avoid competition among species, i.e. below and above ground organs did not overlap. All plots were initially irrigated daily and fertilized twice (Terra Plus N 12+4+6, Compo Expert, Germany) for two months to allow for plant establishment. Plots were then exposed to a dry and an irrigated treatment (32 plots, respectively). In the dry treatment plots, irrigation was discontinued for 10 weeks (3rd August – 9th October 2015), while continuing in the irrigated treatment plots. Soil water potential in the dry treatment plots declined strongly and reached -1.5 MPa, the permanent wilting point in agricultural plants (Veihmeyer & Hendrickson, 1928), within 18 days of

ceasing irrigation (Manuscript 1). At the end of dry treatment, plots were irrigated again and all rainout shelters were removed. For details on the experiment see Manuscript 1.

Trait and performance measurements

In total, 38 traits and four drought performance parameters were assessed (Table 1). For each trait, we had a specific hypothesis for its relation to species comparative drought performance (Table 1), based on a broad range of literature and physiological knowledge. Most traits were assessed under well-watered conditions in the greenhouse, while drought performance, most root traits and their phenotypic plasticity in response to drought were assessed in the common garden experiment. Measurements were taken on various sets of plant in the greenhouse and in the common garden, i.e. different traits were measured on different individuals, but each trait was measured with the same set of plants to ensure comparability across species (see Table S2 for details), and only plants of similar size in the vegetative growth phase were used. Trait and performance measurements were taken on 3-36 individuals per species. For the details about plant sets and replicates for each trait see Table S2.

Traits pertaining to water uptake

Roots are the organ of water uptake from soil. We assessed various root traits in regard to root size, root depth distribution, phenotypic plasticity, and morphology that influence plant access to soil water and thus water uptake (see Table 1).

Root size, i.e. maximum rooting depth, root mass, root total length and root mass ratio, and their phenotypic plasticity in response to drought were assessed in the common garden experiment. In the spring following the experimental drought (see above), plants in eight dry and eight irrigated treatment plots were carefully dug out. Maximum rooting depths ($R_{dep\ dry}$ and $R_{dep\ irr}$) were measured *in situ* for each plant. Roots were carefully washed and plants were oven-dried to assess plant mass, root mass ($R_{mas\ dry}$ and $R_{mas\ irr}$) and root mass ratio ($R_{MR\ dry}$ and $R_{MR\ irr}$; root mass/plant mass). Total root lengths ($R_{TL\ dry}$ and $R_{TL\ irr}$) were calculated by multiplying root mass ($R_{mas\ dry}$ and $R_{mas\ irr}$) and root specific length (R_{SL}, see below). Phenotypic plasticity in response to drought of rooting depth (P_{Rdep}), root mass (P_{Rmas}) and root mass ratio (P_{RMR}) were assessed as the relative distance plasticity index (RDPI, RDPI, Valladares *et al.*, 2006), i.e. the relative trait difference of all pairs of individuals within a species growing in different conditions (the dry and irrigated treatments). This index allows to test the differences

among species (Valladares *et al.*, 2006). The phenotypic plasticity of root total length was not analyzed separately because it is the same as for root mass (P_{Rmas}), see above.

Root depth distribution ($R_{Dep\ Dis}$) was assessed in 35 species from detailed drawings of whole root profiles (Kutschera, 1960; Kutschera *et al.*, 1982), where one 'representative' individual for each species growing under natural conditions or on farmlands was drawn. Root fractions along the continuous soil profiles were analyzed from the scanned figures using ImageJ (Schneider *et al.*, 2012), and fitted with the asymptotic equation: $Y = 1 - R_{Dep\ Dis}{}^{d}$ $Y = 1 - R_{Dep\ Dis}{}^{d}$ where Y is the cumulative fraction of roots from the soil surface to depth d (Gale & Grigal, 1987). Higher values of root depth distribution thus indicate a larger proportion of roots in deeper soil layers. We tested the validity of this approach for assessing root depth distribution by relating them to actual measurements of root biomass per soil layer (up to 40 cm) for plants growing in monocultures under natural conditions in nine species (data from data from Oram *et al.*, 2018). The results of the two methods were significantly positively related (r = 0.71, $p < 0.05$). See Table S1 for the missing species from the measurement.

Root morphological traits, i.e. root diameter (R_{dia}), root specific length (R_{SL}) and root density (R_{den}), were assessed in the greenhouse. Roots were washed and whole roots excluding the tap roots or rhizomes, or a representative subsample of roots were dyed with astra blue and scanned at 400 dpi (Epson Perfection V700 Photo Scanner, Japan) and then oven-dried. Root diameter (R_{dia}), root length and root volume were measured using WinRhizo (Regent Instruments Inc., Canada). Root specific length (R_{SL}) was assessed as the ratio of root length to root mass and root tissue density (R_{den}) as the ratio of root mass to root volume.

Traits pertaining to water transport

The continuation of water transport in leaves is important for maintaining stomatal conductance and photosynthesis under drought (Brodribb & Holbrook, 2003; Bartlett *et al.*, 2016). We assessed leaf vein density and xylem conduit diameter that influence water transport in leaves (Table 1).

Total (VD_{total}), major (VD_{major}) and minor (VD_{minor}) leaf vein density were assessed following Pérez-Harguindeguy *et al.* (2013). One healthy mature leaf per individual was sampled and clarified in 5% w/v NaOH–H_2O and then in a commercial bleach (EAU DE JAVEL, Floreal, Germany) until it was bleached. Leaf was then rinsed in distilled water, dehydrated in an

ethanol dilution series (i.e. 30%, 50%, 70% and 100%) and stained with 1% w/v safranin O (Sigma-Aldrich, USA) in ethanol. Whole leaves were scanned (Brother DCP-195C, Japan) at 1200 dpi to assess major leaf vein density, and then the top, middle and bottom of each leaf were examined with a light microscope (Leitz Dialux 22 EB, Germany) at 5x magnification and pictures were taken (DinoCapture 2.0, Dino-Lite, USA) to assess minor leaf vein density. ImageJ (Schneider *et al.*, 2012) was used for measurements of the leaf vein length and examined area. The first three leaf vein orders were categorized as major leaf veins and higher orders as minor leaf veins, with leaf vein order classification following Ellis *et al.* (1992). Leaf vein density (VD_{total}, VD_{major}, VD_{minor}) was calculated as the leaf vein length per unit area (Pérez-Harguindeguy *et al.*, 2013). Four grass species had very thin leaves, and total and minor leaf vein density in these species were not assessed (see Table S1).

Major ($LV_{dia\ major}$) and minor ($LV_{dia\ minor}$) leaf vein xylem conduit diameter were assessed with leaf transverse sections from the middle of the leaves. One leaf per individual was sectioned by a hand microtome (Allmikro-Mikrotom, Haga, Germany) or by free hands using razor blades. Cross sections were counterstained with astra blue and safranin (Kraus *et al.*, 1998). Images were taken using the light microscope at 40x magnification (see above) and analyzed with ImageJ (Schneider *et al.*, 2012). Xylem conduit was treated as an ellipse. The long (a) and short axes (b) of each conduit in 1-2 major leaf veins and 2-3 minor leaf veins per leaf section were measured with ImageJ (Schneider *et al.*, 2012). The diameter of the conduits was calculated as $LV_{dia} = \frac{\sqrt{a^2+b^2}}{2}$.

Traits pertaining to water storage

Water storage in plant organs can constitute a main water source when water uptake through roots is limited, and has been suggested to determine the time until lethal water potentials are reached in plants under drought (Blackman *et al.*, 2016; Choat *et al.*, 2018). We measured plant tissue water content to assess plants water storage.

Total, shoot and root water content (TWC, SWC and RWC, respectively) of plants were assessed with fully saturated plants in the greenhouse. The fresh and dry above- and below-ground biomass were weighted. Water contents were calculated as WC = (fresh mass – dry mass)/dry mass for each corresponding tissue.

Traits pertaining to water loss

Plant water loss can influence the decline of plant water potentials under drought (Choat *et al.*, 2018). It occurs and is regulated mainly through the stomata and after stomatal closure through the cuticles, We assessed maximum stomatal conductance (g_{max}), stomatal conductance under moderate drought in the morning ($g_{drought}$), leaf water potentials at turgor loss point (Ψ_{tlp}) and at stomatal closure ($\Psi_{stclosure}$), minimum leaf conductance (g_{min}), stomatal density (ST_{den}), stomatal length (ST_{len}), stomatal index (ST_{ind}) and specific leaf area (SLA). These traits are all known or hypothesized to influence plant water loss (Table 1).

Maximum stomatal conductance (g_{max}) was assessed with a portable open gas exchange measurement system equipped with a light source (Li-6400, Li-Cor, USA) in the morning (8:30-11:30 h). Leaves was enclosed into the leaf chamber and exposed to conditions with a photosynthetic photon flux density of 1000 μmol m^{-2} s^{-1}, 400 μmol mol^{-1} CO_2, an air temperature of 22 °C, and a relative humidity of 65%. If leaves smaller than the gas exchange chamber, the relevant leaf area was measured with a leaf area meter (Model 3100, Li-cor, USA), and stomatal conductance was calculated accordingly.

To assess stomatal responses to drought, we measured stomatal conductance under moderate drought conditions ($g_{drought}$) in the common garden experiment in the morning (8:00-11:00h). Measurements were taken on the same plants and the same day as for the midday leaf water potential measurements (see below) using a leaf porometer (AP4 Leaf Porometer, Delta-T Devices Ltd, UK) calibrated each day.

Leaf water potential at turgor loss point (Ψ_{tlp}) was assessed based on the osmotic water potential at full turgor with the 'osmometer' method (Bartlett *et al.*, 2012a; Manuscript 2). A leaf disc (4-mm-diamter) from a rehydrated plant was frozen in liquid nitrogen for two minutes and then sealed in a VAPRO 5500 vapor pressure osmometer (Vapor Model 5600, Wescor, USA). Osmotic water potential at full turgor (π_o) was converted to leaf water potential at turgor loss point (Ψ_{tlp}) with the equation: $\Psi_{tlp} = 0.645 \cdot \pi_o - 0.942$ (Manuscript 2).

Leaf water potential at stomatal closure ($\Psi_{stclosure}$) was assessed in a climate chamber under constant day time conditions (light intensity: 650 μmol m^{-2} s^{-1}, temperature: 24°C and relative humidity: 60%) on plants raised in the greenhouse. After an acclimation period under irrigated conditions, stomatal conductance was measured from 8:00 to 11:30h am using a Li-6400 with

the same leaf chamber conditions for maximum stomatal conductance measurement (see above). Watering was then discontinued and stomatal conductance measured daily during the dry treatment until it reached 20% of its optimal before the dry treatment, which was considered stomatal closure (compare Brodribb & Holbrook, 2003; Brodribb *et al.*, 2003). Leaf water potentials were then measured on the same leaf with a pressure chamber (Model 1505D, PMS Instrument Company, USA).

Minimum leaf conductance (g_{min}) was assessed based on leaf drying curves (Sack *et al.*, 2011). Plants were fully hydrated overnight in the dark. Leaves were cut, the wound was sealed with colorless nail polish (essence, Germany). They were laid out for drying in the climate chamber. The same chamber conditions were used as for the leaf water potential at stomatal closure measurement (see above). The real-time mole fraction of vapor pressure deficiency (mfVPD) was measured using Licor 6400. Leaves were repeatedly weighted at 3-20 mins intervals. Average leaf area (LA) before and after the measurement was calculated. Leaf water loss (WL) was plotted against time (T), and the flat part of the curve after stomatal closure was used to assess minimum leaf conductance (g_{min}). Minimum leaf conductance was calculated as WL/(18*T*mfVPD*LA) following Sack *et al.* (2011), where 18 is molar mass of H_2O.

Stomatal length (ST_{len}), stomatal density (ST_{den}), and a stomatal index (ST_{ind}) were determined from nail polish imprints of the middle of the upper and lower leaf surfaces. Images of a selected field with no dirt and large leaf veins from each side were taken with the light microscope at 20X magnification (see above) and analyzed with ImageJ (Schneider *et al.*, 2012). Stomatal length (ST_{len}) was assessed as the guard cell length. Stomatal density (ST_{den}) was assessed as the total number of stomata on both leaf sides per unit leaf area, and stomatal index (ST_{ind}) was assessed as the total number of stomata relative to the total number of stomata and epidermal cells within the same area in both leaf sides (Salisbury, 1927).

Specific leaf area (SLA) was assessed for three leaves per individual, based on the leaf area measured with a leaf area meter (Model 3100, Li-cor, USA) and leaf mass weighted after oven-drying. Specific leaf area (SLA) was calculated as leaf area per unit leaf mass (Pérez-Harguindeguy *et al.*, 2013).

Traits pertaining to leaf membrane vulnerability

Cell membrane integrity is critical to maintain plant functions such as protein synthesis, photosynthesis and respiration (Bewley, 1979), and plant tissue survival under drought (Guadagno *et al.*, 2017). We measured the leaf electrolyte leakage in response to water stress to assess leaf membrane vulnerability to drought (Martin *et al.*, 1987).

The leaf electrolyte leakage in response to water stress was measured by bench drying fully rehydrated leaves to different water potentials (-0.3 MPa to -10 MPa; method adapted from -0.3 MPa to -10 MPa; method adapted from Martin *et al.*, 1987). Leaf water potentials were measured with a pressure chamber (Model 1505D, PMS Instrument Company, USA). Then, four leaf discs (4-mm diameter) were punched out from each leaf and submerged in 25mL distilled water. The electrical conductivity (EC) of the water was measured initially (EC_0), after 24 hours (EC_{24}) and after boiling for 20 minutes (EC_{total}) with an electrical conductivity meter (PM 2000 set, Carl Roth, Karlsruhe, Germany). Electrolyte leakage was calculated as $(EC_{24}-EC_0)/(EC_{total}-EC_0)\times100$. The relations between leaf water potential and electrolyte leakage were fitted by sigmoidal models. Leaf membrane vulnerability was calculated as leaf water potentials at which 50% ($\Psi_{50\%ML}$) and 80% ($\Psi_{80\%ML}$) electrolyte leakage occur. $\Psi_{50\%ML}$ and $\Psi_{80\%ML}$ were highly correlated ($r = 0.72$, $P < 0.001$) with only few $\Psi_{80\%ML}$ values extrapolated from the models. Yet, $\Psi_{80\%ML}$was used in the analyses considering that high levels of membrane leakage are irreversible and have higher physiological impacts.

Traits pertaining carbon gain

Maintenance of carbon gain is considered important for plants metabolism and growth under drought (McDowell, 2011). We assessed maximum photosynthesis (A_{max}), intrinsic (WUE_{intr}) and integrated (WUE_{inte}) water use efficiency, leaf nitrogen content (N_{mass}) and leaf carbon/nitrogen ratio (C/N) that may influence plant carbon gain under drought (Table1).

Leaf C/N ratio was assessed as C_{mass}/N_{mass} (see below). Maximum photosynthesis (A_{max}) and intrinsic water use efficiency (WUE_{intr}) were assessed simultaneously with maximum stomatal conductance (g_{max}, see above for details). Intrinsic water use efficiency was assessed as A_{max}/g_{max} (Pérez-Harguindeguy *et al.*, 2013).

Integrated water use efficiency (WUE_{inte}) was assessed based on isotope discrimination (Δ) against ^{13}C due to preferential use of ^{12}C during photosynthesis. The abundance of ^{13}C and total leaf carbon (C_{mass}) and nitrogen (N_{mass}) content were analyzed in BayCEER-Laboratory

of Isotope Biogeochemistry with an elemental analyzer (EA-IRMS coupling, Carlo Erba 1108, Milano, Italy) coupled to an isotope ratio mass spectrometer (delta S; Finnigan MAT, Bremen, Germany) via a ConFlo III open-split interface (Finnigan MAT, Bremen, Germany) (Bidartondo *et al.*, 2004). The relative abundance of $\delta^{13}C$ of samples ($^{13}C/^{12}C$ ratios) were expressed with reference to the international Pee Dee Belemnite (PDB) standard based on the equation:

$$\delta^{13}C_{Sample} = \left[\frac{\left(^{13}C/^{12}C\right)_{Sample}}{\left(^{13}C/^{12}C\right)_{PDB}} - 1\right] * 1000.$$

$\delta^{13}C$ isotope discrimination was defined as

$$\Delta = \frac{\delta^{13}C_{air} - \delta^{13}C_{plant}}{1 + \frac{\delta^{13}C_{plant}}{1000}},$$

where $\delta^{13}C_{air}$ = -8‰ (Pérez-Harguindeguy *et al.*, 2013). Following Farquhar and Richards (1984), the isotope discrimination Δ is proportional to the ratio of intercellular (C_i) to ambient (C_a) CO_2 concentrations:

$$\Delta \cong a + (b - a)\frac{C_i}{C_a},$$

where a (4.4‰) is the fractionation during the diffusion of CO_2 through the stomata, b (27‰) is the fractionation associated with carboxylation (Farquhar & Richards, 1984), C_a was from direct measurements of atmospheric CO_2 concentrations (Dlugokencky & Tans, 2017). The integrated water use efficiency, the ratio of net photosynthesis (A) to conductance for water vapour g_w was estimated from Δ:

$$\mathrm{WUE_{inte}} = \frac{A}{g_w} = \frac{C_a}{1.6}\frac{(b-\Delta)}{(b-a)}$$ (Seibt *et al.*, 2008).

Drought performance parameters

Whole-plant drought resistance of both survival and growth, and midday leaf water potentials under drought were assessed experimentally based on the performances in the dry and irrigated treatments in the common garden drought experiment.

Species survival was assessed based on above-ground biomass in the spring after the experiment to allow for potential recovery. Survival in dry and irrigated plots (S_{dry} and S_{irr}, respectively) were calculated as $S = N_{end}/N_{initial} * 100$, where N_{end} and $N_{initial}$ are the number of individuals at the beginning and the end of the experiment.

Relative growth rates were assessed based on the projected leaf area in six drought (RGR_{dry}) and six irrigated (RGR_{irr}) treatment plots in the first week and sixth week of dry treatment as $RGR = (LA_1 - LA_2)\ LA_1^{-1}(T_2 - T_1)^{-1}$, where LA_1 and LA_2 is projected green leaf area in the first week (T_1) and the sixth week (T_2) of the dry treatment.

Whole-plant drought resistance of survival ($DR_{survival}$) and growth (DR_{growth}) were assessed as response ratios in the drought relative to irrigated treatment: $DR_{survival} = S_{dry}/S_{irr}$ and $DR_{growth} = RGR_{dry}/RGR_{irr}$, where RGR_{dry} and RGR_{irr} were the median relative growth rates in the dry treatment plots and in the irrigated treatment plots, respectively (Manuscript 1).

Midday leaf water potentials were assessed under moderate drought, i.e. when most plants had started wilting after eight days of the dry treatment. Leaf water potentials were measured in eight randomly selected dry treatment plots using leaf cutter psychrometers (Merrill Specialty Equipment, USA) and a PSYPRO™ water potential system (Wescor, Inc., USA). All species in one plot were sampled within one day from 11:30 to 12:30h to ensure comparability across species and avoid any bias introduced by sampling date. Psychrometer measurements were taken after five hours equilibration (Manuscript 2).

Species association to habitat dryness (D_{ass}) was based on Ellenberg's soil moisture indicator values (Table S1; Ellenberg *et al.*, 1991). Ellenberg's indicator values categorize species based on extensive non-systematic field observations (Ellenberg *et al.*, 1991). Previous studies have shown that M-values were highly correlated with actual quantification of habitat water availability in Europe and can be treated as continuous values (Diekmann, 2003; Schaffers & Sýkora, 2009; Bartelheimer & Poschlod, 2016). We multiplied Ellenberg's soil moisture indicator values (M-values) with -1 so that higher values indicate higher association to drier habitats, consistent with the direction of values of whole-plant drought resistance of survival and growth (see above). 11 species without specific moisture requirements according to Ellenberg's indicator values (M-value = x) were excluded from analyses concerning to habitat association.

Statistics:

Normality of data distribution was tested with the Shapiro–Wilk tests and when needed, data were log-transformed to increase normality. One-way ANOVAs were used to test for species differences in each trait across all species, as well as within forbs and grasses, separately.

To evaluate coordination and trade-offs among traits, we first tested pairwise correlations between all traits using Pearson's correlations. We additionally used Principle components analysis (PCA) to evaluate the main axes of trait covariations. Three traits, i.e. root depth distribution ($R_{dep\ dis}$), and total and minor leaf vein density (VD_{total} and VD_{minor}), were excluded from the PCA analysis and the following analyses because of incomplete datasets (see above).

To assess the importance of traits for drought performance parameters, i.e. whole-plant drought resistance of survival, whole-plant drought resistance of growth, midday leaf water potential under moderate drought and species association to habitat dryness, we used a permutation-based random forest algorithm. It evaluates the contribution of each trait to the change in the prediction accuracy of the model when the trait values are randomly permuted (Genuer *et al.*, 2010; Hapfelmeier & Ulm, 2013). Unlike linear models, the evaluation of trait importance with random forest model does not specify a particular structure on the data, assesses both direct and indirect effects of traits on drought performance parameters (Jones & Linder, 2015), and can deal with traits that are highly correlated with each other (Strobl *et al.*, 2008). The empirical distribution of trait importance under the universal null hypothesis (H_0) that a trait made no contribution to improving model accuracy were assessed using a permutation approach (Hapfelmeier & Ulm, 2013). Significances of all trait importance were then evaluated at α = 0.05 with Bonferroni adjustment to reduce type I error for multiple statistical tests (Hapfelmeier & Ulm, 2013). Bonferroni adjustment is advocated where multiple significances are tested under the same null hypothesis (H0; Hapfelmeier & Ulm, 2013; Armstrong, 2014). For other significance tests, we had specific individual hypotheses for each test. Their results and discussions thus are based on unadjusted values, but we additionally presented results with Bonferroni adjustment at α = 0.05. Spearman's rank correlations were used to test if the rank of trait importance differed between different drought performance parameters. We additionally used Pearson's coefficients to evaluate the directions and strength of relations between individual traits and drought performance parameters.

T-tests were used to test for differences between forbs and grasses in each individual trait and in drought performance parameters. Differentiation of traits between forbs and grasses was additionally analyzed in multidimensional trait space based on PCA (see above). We analyzed the 95% confidence interval of the trait averages in forbs and grasses along PC1 and PC2. To test if the importance of traits varies across life forms, trait importance and its significance were assessed separately for forbs and grasses using the permutation-based random forest algorithms (see above). Spearman's rank correlations were then used to test if the rank of trait importance differs between forbs and grasses within and across drought performance parameters.

All analyses were done using R (R Core Team, 2018). The permutation-based random forest algorithm was employed with extendedForest package (Smith *et al.*, 2011) based on the code provided in Hapfelmeier and Ulm (2013).

Table 1 List of the traits and drought performance parameters, abbreviations, and functional significance of traits for and their hypothesized relations to drought performances.

Traits	Abbreviation	Unit	Significance	Hypotheses
Performance under drought				
whole-plant drought resistance of survival	$DR_{survival}$	%/%		
whole-plant drought resistance of growth	DR_{growth}	%/%		
midday leaf water potential	MWP	MPa		
association to habitat dryness (Ellenberg's indicator values)	D_{ass}	unitless		
Water uptake				
maximum rooting depth dry	$R_{dep\ dry}$	cm	High maximum rooting depth increases plants access to deep soil where soil water is more liable (Barkaoui *et al.*, 2016)	+
maximum rooting depth irrigated	$R_{dep\ irr}$	cm		+
root depth distribution	$R_{dep\ dis}$	unitless	High root depth distribution indicates high fraction of biomass allocated to deep roots (Jackson *et al.*, 1996)	+
root mass dry	$R_{mas\ dry}$	g	High root mass indicates a large, deep and robust root system and a high capacity of root acquisition of water (Fort *et al.*, 2013)	+
root mass irrigated	$R_{mas\ irr}$	g		+

root total length dry	$R_{TL\ dry}$	m		+
root total length irrigated	$R_{TL\ irr}$	m	High root total length increases root volume and surface area in contact with and for the exploration of soil water (Comas *et al.*, 2013)	+
root mass ratio dry	$R_{MR\ dry}$	g g^{-1}		+
root mass ratio irrigated	$R_{MR\ irr}$	g g^{-1}	High root mass ratio indicates high biomass allocated to water uptake relative to light interception and transpiration area (Comas *et al.*, 2013)	+
root diameter	R_{dia}	mm		-
root specific length	R_{SL}	m g^{-1}	Low root diameter and/or root tissue density enable high root length per biomass invested, i.e. high specific root length, increasing root exploration of soil water per unit of carbon allocated (Comas *et al.*, 2013; Ma *et al.*, 2018)	+
root tissue density	R_{den}	g cm^{-3}		-
phenotypic plasticity of rooting depth	P_{RDep}	cm cm^{-1}		+
phenotypic plasticity of root mass	P_{RMas}	g g^{-1}	High phenotypic plasticity of root traits allows plants to increase their water uptake capacity in response to temporal water stress (Comas *et al.*, 2013; Bardgett *et al.*, 2014)	+

phenotypic plasticity of root mass ratio	P_{RMR}	$(g\ g^{-1})/(g\ g^{-1})$		+
Water transport				
total leaf vein density	VD_{total}	$mm\ mm^{-2}$		+
major leaf vein density	VD_{major}	$mm\ mm^{-2}$	High leaf vein density can provide hydraulic redundancy that buffer disruption of the hydraulic system caused by drought-induced embolism (Sack & Scoffoni, 2013)	+
minor leaf vein density	VD_{minor}	$mm\ mm^{-2}$		+
major leaf vein xylem conduit diameter	$LV_{dia\ major}$	µm	Low conduit diameter is associated with high resistance to drought-induced embolism (Davis *et al.*, 1999; Scoffoni *et al.*, 2017)	-
minor leaf vein xylem conduit diameter	$LV_{dia\ minor}$	µm		-
Water storage				
total water content	TWC	%		+
shoot water content	SWC	%	High water storage can constitute a main water source when soil water uptake through roots is limited (Pineda-Garcia *et al.*, 2013; Blackman *et al.*, 2016)	+
root water content	RWC	%		+
Water loss				

maximum stomatal conductance	g_{max}	$mmol_{H2O}$ m^{-2} s^{-1}		-
stomatal conductance under moderate drought in the morning	$g_{drought}$	$mmol_{H2O}$ m^{-2} s^{-1}	Low stomatal conductance reduces water loss through the stomata and slows the decline of plant water potentials under drought down (Choat *et al.*, 2018)	-
minimum leaf conductance	g_{min}	$mmol_{H2O}$ m^{-2} s^{-1}	Low minimum leaf conductance reduces water loss through cuticle and leaked stomata (Brodribb *et al.*, 2014)	-
leaf water potential at turgor loss point	ψ_{tlp}	MPa	High leaf water potential at turgor loss point enables plants to lose turgor early under drought, leading to stomatal closure (Brodribb & Holbrook, 2003), reduced leaf area (Scoffoni *et al.*, 2014) and shrinkage of cuticle waxes reducing cuticular conductance (Boyer, 2015)	+
leaf water potential at stomatal closure	$\psi_{stclosure}$	MPa	High leaf water potential at stomatal closure enables plants to close stomata quickly in response to drought (Klein, 2014)	+
stomatal density	ST_{den}	number mm^{-2}		+
stomatal index	ST_{ind}	%	Small stomata with high density and high stomatal index confer plants the capacity to rapidly reduce stomatal conductance in response to drought while attaining high stomatal conductance under optimal conditions (Salisbury, 1927; Franks *et al.*, 2009)	+
stomatal length	ST_{len}	µm		-
specific leaf area	SLA	cm^2 g^{-1}	Low specific leaf area is considered indicative of low transpiration area per leaf mass and therefore might lead to reduced water loss (Reich *et al.*, 1999; Poorter *et al.*, 2009)	-
membrane vulnerability				

leaf water potential at 80% membrane leakage	$\psi_{80\%ML}$	MPa	Low leaf water potential at 80% membrane leakage enables plants tissue to resist severe dehydration while keeping cell integrity and functions	-
carbon gain				
maximum photosynthesis	A_{max}	μmol_{CO2} m^{-2} s^{-1}	High maximum photosynthesis increases plants carbon gain and may thus enhance carbon storage under drought and avoid or delay carbon starvation (McDowell *et al.*, 2008)	+
intrinsic water use efficiency	WUE_{intr}	μmol_{CO2} m^{-2} s^{-1} /mol_{H2O} m^{-2} s^{-1}	High water use efficiency indicates high carbon gain per water transpired (Farquhar *et al.*, 1982; Farquhar & Richards, 1984) and may reduce water loss through stomata under drought	+
integrated water use efficiency	WUE_{inte}	µmol mol-1		+
leaf nitrogen content	N_{mass}	% N	High leaf nitrogen content increases plants photosynthesis capacity (Wright *et al.*, 2004) and water use efficiency (Farquhar & Richards, 1984)	+
leaf carbon/nitrogen ratio	C/N	$g\ g^{-1}$	Low leaf carbon/nitrogen ratio indicates high proportion of leaf nitrogen content	-

Trait are categorized into groups pertaining to water uptake, water transport, water storage, water loss, membrane vulnerability and carbon gain. Hypothesized directions of relations to drought performances are indicated by + and – as positive and negative relation, respectively.

Results

Coordination and trade-offs among traits

All traits significantly differed among species across all species, as well as within forbs and within grasses (Table 2). Many individual traits correlated within and across processes, i.e., water uptake, water transport, water storage, water loss, membrane vulnerability and carbon gain, showing that many coordination and trade-offs existed among traits. However, while traits clearly did not vary independently, overall the relations were weak and no clear main dimensions of trait variation emerged (Fig. 1, Fig. 2, Fig. 3).

Within traits promoting water uptake, maximum rooting depth ($R_{dep\ dry}$ and $R_{dep\ irr}$), root mass ($R_{mas\ dry}$ and $R_{mas\ irr}$) and root total length ($R_{TL\ dry}$ and $R_{TL\ irr}$) were positively coordinated in both dry and irrigated treatments, and species with high maximum rooting depth in dry treatment ($R_{dep\ dry}$) had a more pronounced distribution of roots to deep soil layers ($R_{dep\ dis}$, Fig. 1, Fig. 2 A and B). At the same time high maximum rooting depth in dry treatment ($R_{dep\ dry}$) was coordinated with high phenotypic plasticity of rooting depth (P_{RDep}) in response to drought, indicating that phenotypic plasticity was key for achieving deep roots under drought (Fig. 2 C). Root morphology was limited by strong trade-offs, where high root specific length (R_{SL}, i.e. high length per biomass) was associated with low root diameter (R_{dia}) and low root tissue density (R_{den}, Fig. 2 D and E).

Within traits related to water transport, species with high density of major leaf veins (VD_{major}) had a low density of minor leaf veins (VD_{minor}, Fig. 2 L) suggesting a trade-off in the construction of the leaf water transport system, while the vessel diameters of major ($LV_{dia\ major}$) and minor leaf veins ($LV_{dia\ minor}$) were positively correlated with each other (Fig. 2 K).

Table 2 Summary statistics of traits: (a) the mean ± standard error across all 41 species (with minimum and maximum species averages in parenthesis), (b) the species effect size (i.e. the explained variance by species identity; one-way ANOVA), the mean ± standard error within (c) forbs and (d) grasses (with respective species effect size in parenthesis) and (e) the significance of the differences between forbs and grasses (t-tests), with the direction of significant differences indicated by < and >.

Traits	(a) mean all species	(b) species effect	(c) mean forbs		(d) mean grasses	(e) life form effect
Performance under drought						
$DR_{survival}$	0.91 ± 0.02		0.93 ± 0.03		0.90 ± 0.03	n.s.
DR_{growth}	(0.46, 1.03) 0.65 ± 0.02		0.65 ± 0.04		0.64 ± 0.01	n.s.
MWP	(0.21, 1.03) -2.42 ± 0.16	0.62***	-1.86 ± 0.15	>	-2.95 ± 0.23	***†
D_{ass}	(-5.68, -1.10) 5.23 ± 0.22		(0.64***) 5.18 ± 0.30		(0.50***) 5.31 ± 0.33	n.s.
	(3, 7)					
Water uptake						
$R_{dep\ dry}$	43.77 ± 1.54	0.39***	46.93 ± 2.60	>	40.75 ± 1.48	*
$R_{dep\ irr}$	(29.00, 74.17) 33.71 ± 0.78	0.29***	(0.43***) 32.96 ± 1.28		(0.26***) 34.42 ± 0.93	n.s.
$R_{dep\ dis}$	(20.67, 45.17) 0.94 ± 0.01		(0.30***) 0.94 ± 0.01		(0.26***) 0.94 ± 0.005	n.s.
$R_{mas\ dry}$	(0.86, 0.99) 6.25 ± 0.89	0.45***	6.46 ± 1.36		6.04 ± 1.20	n.s.
$R_{mas\ irr}$	(0.57, 25.85) 7.32 ± 1.20	0.60***	(0.38***) 4.72 ± 0.99	<	(0.58***) 9.78 ± 2.02	*
$R_{TL\ dry}$	(1.10, 45.00) 1783 ± 278	0.73***	(0.51***) 1098 ± 70.8		(0.60***) 2436 ± 219	n.s.
$R_{TL\ irr}$	(13.15, 21902) 2177 ± 340	0.67***	(0.51***) 836 ± 60.0	<	(0.76***) 3456 ± 201	*
$R_{MR\ dry}$	(52.99, 14774) 0.66 ± 0.02	0.42***	(0.53***) 0.64 ± 0.04		(0.57***) 0.68 ± 0.02	n.s.
$R_{MR\ irr}$	(0.37, 0.94) 0.69 ± 0.03	0.67***	(0.57***) 0.67 ± 0.05		(0.19***) 0.70 ± 0.03	n.s.
R_{dia}	(0.21, 0.97) 0.38 ± 0.06	0.93***	(0.71***) 0.42 ± 0.004	>	(0.58***) 0.34 ± 0.004	**
R_{SL}	(0.26, 0.59) 173 ± 4.28	0.93***	(0.71***) 104 ± 4.33	<	(0.53***) 239 ± 10.1	*
R_{den}	(16.87, 983.96) 0.11 ± 0.02	0.86***	(0.68***) 0.14 ± 0.004	>	(0.56) 0.08 ± 0.002	**
	(0.02, 0.27)		(0.64***)		(0.57***)	

P_{RDep}	0.18 ± 0.01	0.18***	0.21 ± 0.01	>	0.16 ± 0.01	***
	(0.10, 0.32)		(0.19***)		(0.21***)	
P_{RMas}	0.45 ± 0.02	0.13***	0.45 ± 0.03		0.45 ± 0.02	n.s.
	(0.25, 0.79)		(0.23***)		(0.26***)	
P_{RMR}	0.17 ± 0.01	0.25***	0.19 ± 0.02		0.14 ± 0.01	n.s.
	(0.06, 0.47)		(0.38***)		(0.27***)	
Water transport						
VD_{total}	3.28 ± 0.23	0.95***	2.70 ± 0.26	<	3.97 ± 0.33	**
	(1.58, 7.72)		(0.94***)		(0.87***)	
VD_{major}	2.29 ± 0.23	0.95***	1.16 ± 0.14	<	3.36 ± 0.26	***†
	(0.48, 6.87)		(0.94***)		(0.87***)	
VD_{minor}	1.02 ± 0.15	0.95***	1.53 ± 0.22	>	0.41 ± 0.05	***†
	(0.19, 4.93)		(0.94***)		(0.87***)	
$LV_{dia\ major}$	141.10 ± 5.94	0.82***	122.43 ± 8.76	<	158.88 ± 5.98	**
	(51.48, 222.81)		(0.82***)		(0.66***)	
$LV_{dia\ minor}$	5.09 ± 0.16	0.64***	4.50 ± 0.16	<	5.66 ± 0.21	***†
	(3.03, 7.38)		(0.45***)		(0.60***)	
Water storage						
TWC	84.14 ± 0.55	0.81***	84.52 ± 0.91		83.77 ± 0.64	n.s.
	(75.36, 93.06)		(0.65***)		(0.65***)	
SWC	83.35 ± 0.66	0.89***	84.86 ± 0.85	>	81.92 ± 0.91	*
	(74.68, 94.61)		(0.86***)		(0.89***)	
RWC	85.39 ± 0.65	0.70***	84.78 ± 1.22		85.97 ± 0.52	n.s.
	(73.02, 96.16)		(0.88***)		(0.32 n.s.)	
Water loss						
g_{max}	492.8 ± 67.39	0.74***	475.8 ± 106.1		509.0 ± 86.73	n.s.
	(138.9, 1992.2)		(0.79***)		0.68***	
$g_{drought}$	194.7 ± 24.26	0.35***	284.5 ± 38.12	>	109.1 ± 15.27	***†
	(29.45, 611.3)		(0.26**)		(0.29***)	
g_{min}	5.03 ± 0.32	0.58***	6.13 ± 0.45	>	3.99 ± 0.34	***†
	(2.09, 10.35)		(0.44***)		(0.64***)	
ψ_{tlp}	-1.79 ± 0.03	0.61***	-1.73 ± 0.03	>	-1.85 ± 0.04	*
	(-2.30, -1.49)		(0.68***)		(0.54***)	
$\psi_{stclosure}$	-2.59 ± 0.14	0.45*	-2.16 ± 0.15	>	-3.00 ± 0.18	***†
	(-5.10, -1.16)		(0.44 n.s.)		(0.37 n.s.)	
ST_{den}	150.8 ± 16.80	0.82***	185.5 ± 27.93	>	117.7 ± 16.87	*
	(15.16, 438.48)		(0.78***)		(0.84***)	
ST_{ind}	15.52 ± 0.53	0.54***	16.11 ± 0.83		14.96 ± 0.67	n.s.
	(6.32, 23.83)		(0.56***)		(0.48***)	
ST_{len}	34.43 ± 1.60	0.77***	35.59 ± 2.55		33.32 ± 1.99	n.s.
	(21.73, 64.80)		(0.80***)		(0.74***)	

SLA	187.7 ± 10.10	0.80***	194.3 ± 16.70	180.9 ± 12.03	n.s.
	(80.61, 421.5)		(0.85***)	(0.72***)	
membrane vulnerability					
$\psi_{80\%ML}$	-4.88 ± 0.44		-4.91 ± 0.84	-4.86 ± 0.32	n.s.
	(-1.58, -17.88)				
carbon gain					
A_{max}	11.98 ± 0.44	0.79***	12.46 ± 0.76	11.53 ± 0.47	n.s.
	(5.60, 20.06)		(0.84***)	(0.62***)	
WUE_{intr}	37.68 ± 2.55	0.70***	40.15 ± 3.52	35.32 ± 3.69	n.s.
	(6.55, 73.63)		(0.62***)	(0.73***)	
WUE_{inte}	21.98 ± 0.22	0.67***	21.90 ± 0.26	22.05 ± 0.36	n.s.
	(19.19, 26.49)		(0.73***)	(0.64***)	
N_{mass}	1.90 ± 0.11	0.73***	2.04 ± 0.19	1.73 ± 0.13	n.s.
	(0.89, 4.31)		(0.78***)	(0.61***)	
C/N	27.76 ± 1.46	0.70***	25.72 ± 2.00	29.70 ± 2.07	n.s.
	(10.39, 52.09)		(0.71***)	(0.67***)	

Significances are given as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s. $p > 0.05$. Note † indicates that life form effects are still significant after Bonferroni adjustment at $\alpha = 0.05$. For details on trait measurements and calculations see methods and Table S2. Root depth distribution has only one value available per species, and species association to dry habitats, whole-plant drought resistance of survival and growth, and membrane stability were assessed at species level, therefore species effects could not be analyzed for these variables.

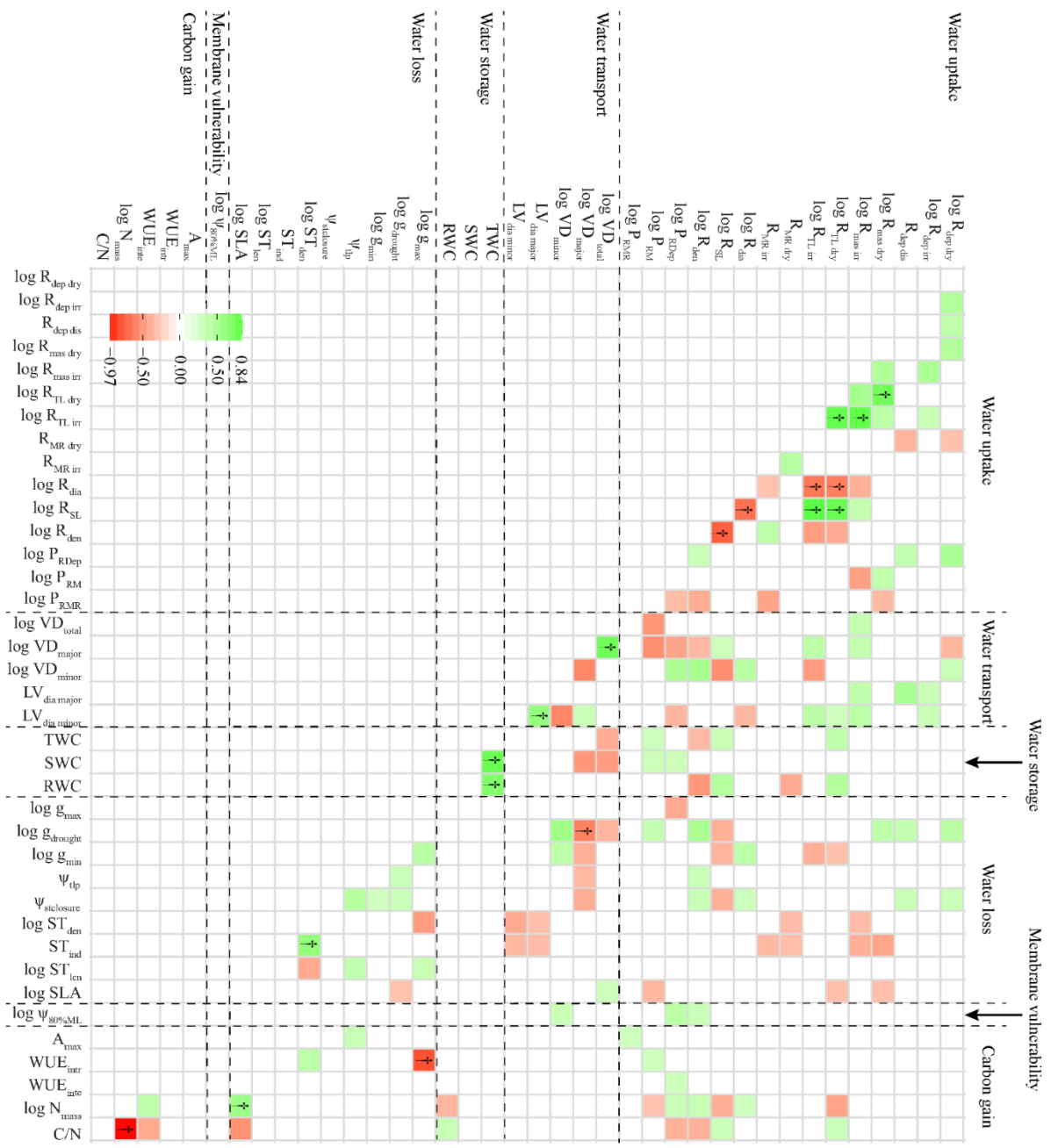

Water uptake
Water transport
Water storage
Water loss
Membrane vulnerability
Carbon gain
−0.97
−0.50
0.00
0.50
0.84

Fig. 1 Correlations among traits. Green and red indicate positive and negative correlations, respectively, the color intensity indicates the strength of the correlations, i.e. Pearson's coefficients. Shown are significant correlations at $P < 0.05$ before and after Bonferroni adjustment (marked with †). Traits are grouped into groups pertaining to water uptake, water transport, water storage, water loss, membrane vulnerability and carbon gain processes. Note that trait correlations occurred both within and across processes. For detailed results of correlation analyses see supplementary file 1 and see Fig. 2.

Within traits pertaining to water loss, species losing leaf cell turgor at high leaf water potentials (Ψ_{tlp}) also closed stomata at high leaf water potentials ($\Psi_{stclosure}$), with turgor loss considerably preceding stomatal closure (Fig. 2 G). Unexpectedly, leaf water potentials at turgor loss point and at stomatal closure were positively correlated with stomatal conductance under drought ($g_{drought}$, Fig. 2 H and I), i.e. species that lost turgor and closed stomata early at high water potentials at the same time were able to maintain high stomatal conductance under moderate drought in the morning. Low specific leaf area (SLA), reflecting low transpirational area per leaf mass, was related to high stomatal conductance under moderate drought in the morning ($g_{drought}$, Fig. 1). High maximum stomatal conductance (g_{max}) was associated with high minimum leaf conductance (g_{min}, Fig. 2F), suggesting a trade-off between the potential for high transpiration rate under well-watered conditions and the capacity to minimize leaf water loss through stomatal closure. High g_{max} was also associated with low stomatal density (ST_{den}) but large stomatal pore length (ST_{len}; Fig. 1).

Within traits associated with carbon gain, species with high leaf nitrogen content (N_{mass}) had high integrated water use efficiency (WUE_{inte}, Fig. 2 M) and a low leaf C/N ratio (Fig. 1).

Coordination and trade-offs also existed across different processes and different plant organs (Fig. 1, Fig. 2). Species with high maximum rooting depth in dry conditions ($R_{dep\ dry}$) facilitating water uptake had high minor leaf vein density (VD_{minor}) to transport and distribute water (Fig. 1) and closed stomata at high leaf water potentials ($\Psi_{stclosure}$) to minimize water loss (Fig. 2 N) but unexpectedly maintained high leaf conductance under moderate drought in the morning ($g_{drought}$, Fig. 2 O). High total root length in dry conditions ($R_{TL\ dry}$), high specific root length (R_{SL}) and low root tissue density (R_{den}) were coordinated with high root water content (RWC; Fig. 1). High phenotypic plasticity of rooting depth (P_{RDep}) and root mass (P_{RMas}) were coordinated with shoot water content (SWC), suggesting that high water storage facilitated root growth under dry conditions (Fig. 1). High leaf water potential at turgor loss point (Ψ_{tlp}) was

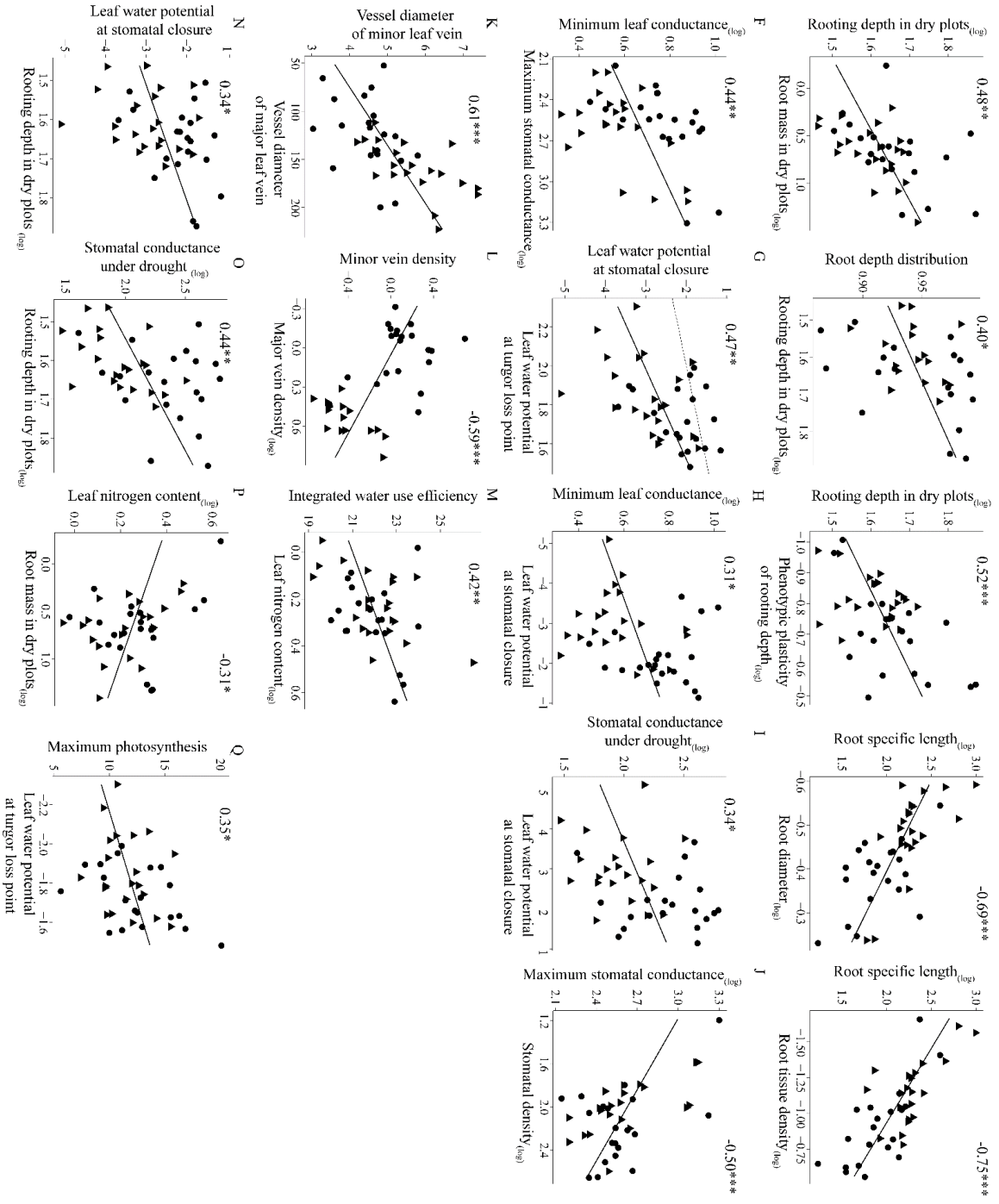

Correlations within traits pertaining to water uptake
forb
grass
Correlations within traits pertaining to water loss
Correlations within traits pertaining to water transport or carbon gain
Correlations across traits pertaining to water uptake, water loss and carbon gain
A
Rooting depth in dry plots(log)
Root mass in dry plots(log)
0.48**
B
Root depth distribution
Rooting depth in dry plots(log)
0.40*
C
Rooting depth in dry plots(log)
Phenotypic plasticity of rooting depth(log)
0.52***
D
Root specific length(log)
Root diameter(log)
-0.69***
E
Root specific length(log)
Root tissue density(log)
-0.75***
F
Minimum leaf conductance(log)
Maximum stomatal conductance(log)
0.44**
G
Leaf water potential at stomatal closure
Leaf water potential at turgor loss point
0.47**
H
Minimum leaf conductance(log)
Leaf water potential at stomatal closure
0.31*
I
Stomatal conductance under drought(log)
Leaf water potential at stomatal closure
0.34*
J
Maximum stomatal conductance(log)
Stomatal density(log)
-0.50***
K
Vessel diameter of minor leaf vein
Vessel diameter of major leaf vein
0.61***
L
Minor vein density
Major vein density(log)
-0.59***
M
Integrated water use efficiency
Leaf nitrogen content(log)
0.42**
N
Leaf water potential at stomatal closure
Rooting depth in dry plots(log)
0.34*
O
Stomatal conductance under drought(log)
Rooting depth in dry plots(log)
0.44**
P
Leaf nitrogen content(log)
Root mass in dry plots(log)
-0.31*
Q
Maximum photosynthesis
Leaf water potential at turgor loss point
0.35*

Fig. 2 Selected pairwise correlations among traits to exemplify the coordination and trade-offs. Shown are associations among traits pertaining to water uptake (A)-(E), to water loss (F)-(J), and to water transport or carbon gain processes (K)-(M). (N)-(Q) show coordination and trade-offs among traits pertaining to different processes. Given are Pearson's correlation coefficients and corresponding significant as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Note in (G) the 1:1 dashed line was added. For units of the traits see Table 1.

associated with high maximum photosynthesis (Amax, Fig. 2 Q) allowing high carbon gain before turgor loss. Leaf membrane vulnerability, i.e. leaf water potential at 80% membrane leakage (80%ML), was significantly related to root tissue density (Rden), phenotypic plasticity of rooting depth (PRDep) and minor leaf vein density (VDminor, Fig. 1).

Despite the multitude of trait correlations, traits for some processes varied independently (Fig. 1). For example, traits pertaining to water storage were not related to any water loss traits or to membrane vulnerability, and traits pertaining to water transport were unrelated to membrane vulnerability and carbon gain (Fig. 1). The independence of trait variations also existed for the 'fast to slow' economics spectrum traits (Fig. 1). Only a few significant correlations were found, namely root specific length (RSL) was negatively correlated with root tissue density (RDen, see above). Leaf nitrogen content (Nmass) was significantly positively correlated with specific leaf area (SLA) and root tissue density (RDen), and negatively with root specific length but the directions with the latter two were opposite to the 'fast to slow' economics spectrum expectation. No further significant correlations among them including maximum photosynthesis (Amax) were supported with our data.

Consistently, the PCA analysis showed that the variance explained by the first three axes was low (only 43%, Fig. 3, Table 2), although these axes captured some pronounced trait covariations within and across processes. Further, only traits pertaining to water uptake and water transport and stomatal traits had high loadings along the axes, other traits loadings were relatively low (Table 2). These results indicate that the variation of traits related to drought resistance is not coordinated along a few main axes, but that many orthogonal dimensions of trait variation exist – consistent with the many correlations and independence among individual traits (see above).

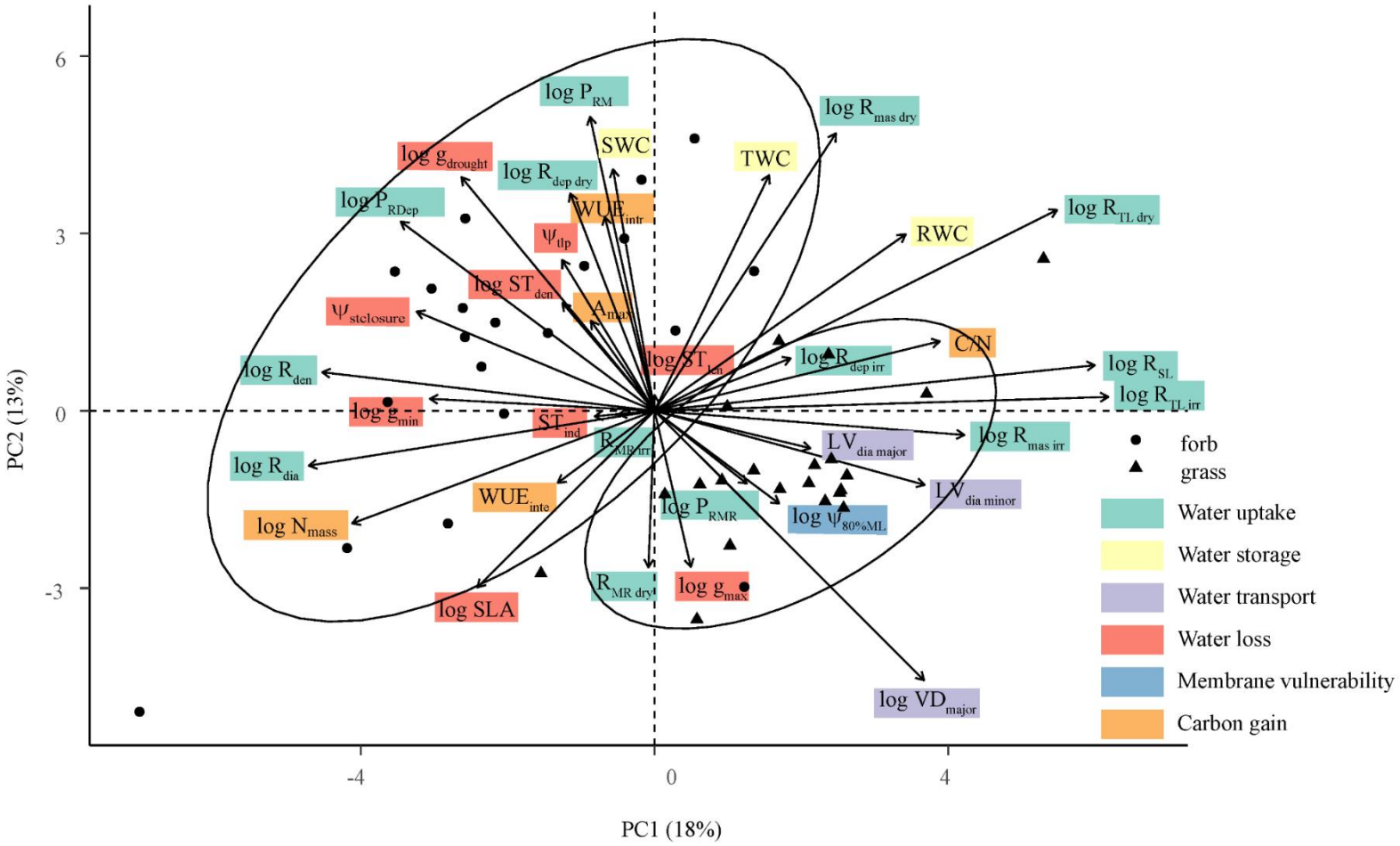


Fig. 3 Results of Principle components analyses (PCA), i.e. trait and species positions along PC1 and PC2. Traits were colored according to the processes they pertain to. Species were categorized by their life forms, and ellipses indicate 95% confidence intervals of the mean in each life form. For detailed trait loadings on PC1 and PC2 see Table 2. Root depth distribution, and total and minor leaf vein density were excluded in this analysis because they have incomplete datasets.

Table 2 Trait loadings on main PCA axes, i.e. PC1, 2 and 3, and variances captured by these axes.

traits	PC1 (18%)	PC2 (13%)	PC3 (12%)
Water uptake			
$\log R_{Dep\ dry}$	-0.16	0.52	0.30
$\log R_{Dep\ irr}$	0.26	0.13	0.46
$\log R_{Mas\ dry}$	0.35	**0.67**	0.43
$\log R_{Mas\ irr}$	**0.60**	-0.06	0.50
$\log R_{TL\ dry}$	-0.01	-0.38	0.30
$\log R_{TL\ irr}$	-0.07	-0.01	0.33
$R_{MR\ dry}$	**0.78**	0.48	0.11
$R_{MR\ irr}$	**0.88**	0.03	0.15
$\log R_{Dia}$	**-0.67**	-0.13	-0.01
$\log R_{SL}$	**0.85**	0.11	-0.24
$\log R_{Den}$	**-0.64**	0.09	0.47
$\log PRDep$	-0.49	0.46	-0.05
$\log PRM$	-0.13	**0.71**	-0.07
$\log PRMR$	0.18	-0.18	-0.12
Water transport			
$\log VD_{major}$	0.52	**-0.65**	-0.13
$LV_{Dia\ major}$	0.30	-0.09	**0.60**
$LV_{Dia\ minor}$	0.52	-0.18	0.47
Water storage			
TWC	0.22	0.57	-0.23
SWC	-0.08	0.58	0.06
RWC	0.49	0.43	-0.44
Water loss			
$\log g_{max}$	0.07	-0.38	0.49
$\log g_{drought}$	-0.37	0.56	0.37
$\log g_{min}$	-0.44	0.03	0.24
ψ_{tlp}	-0.18	0.36	0.36
$\psi_{stclosure}$	-0.46	0.24	0.32
$\log ST_{Den}$	-0.18	0.26	**-0.67**
ST_{Ind}	-0.12	-0.01	**-0.70**
$\log ST_{Len}$	0.00	0.04	0.39
$\log SLA$	-0.34	-0.43	-0.01
Membrane stability			
$\log \psi_{80\%ML}$	0.24	-0.22	-0.17
Carbon gain			
A_{max}	-0.12	0.22	0.26
WUE_{Intr}	-0.10	0.47	-0.44
WUE_{Inte}	-0.19	-0.17	0.03
$\log N_{mass}$	-0.59	-0.27	0.02
C/N	0.55	0.17	-0.08

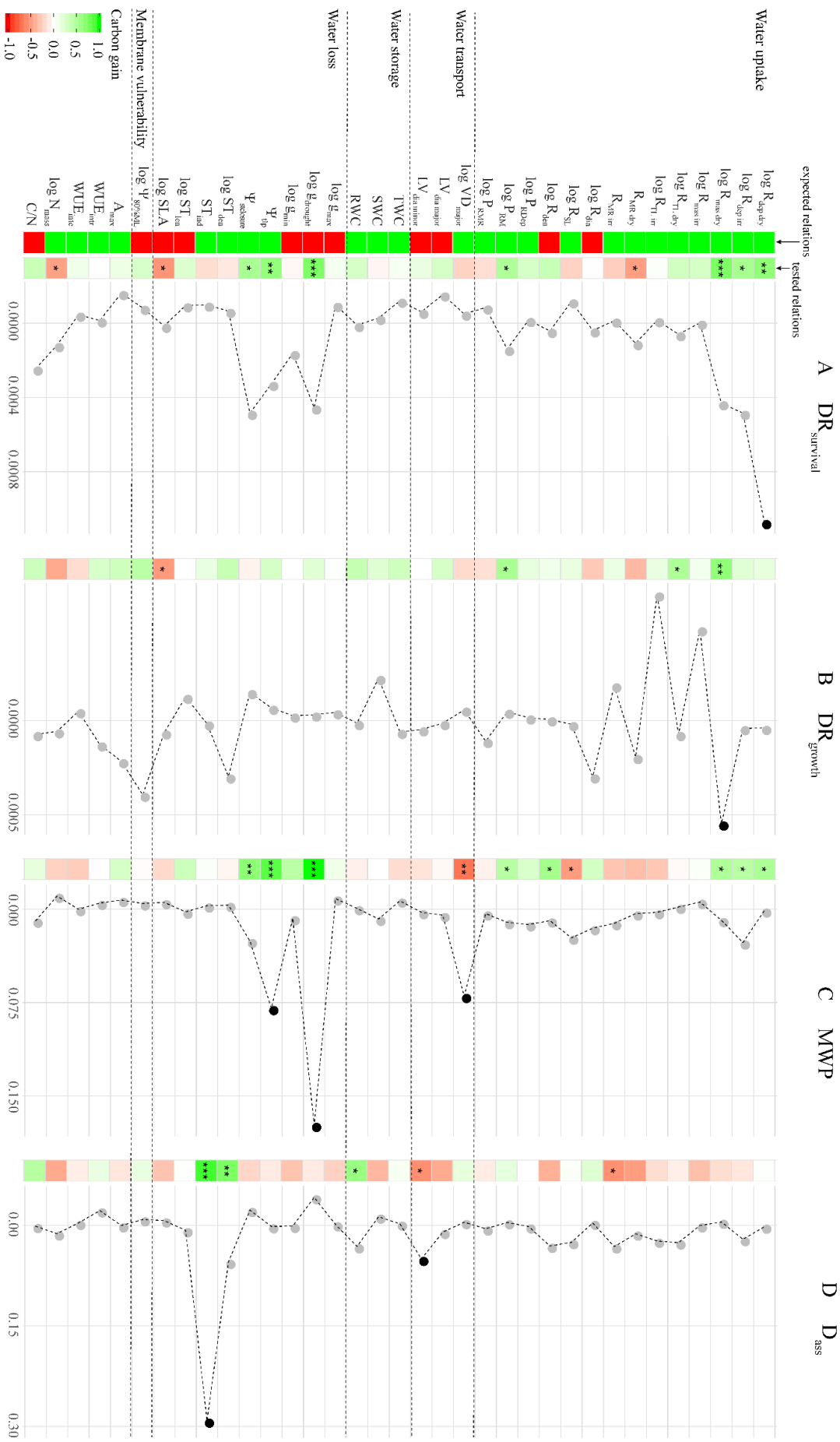
Highlighted are trait loadings ≥ 0.6 or ≤ -0.6, indicating that the PCA axis captures large variations in these traits.

The importance of traits for drought performance parameters across all species

Overall, various individual traits showed significant relations with species drought performances, i.e. whole-plant drought resistance of survival, whole-plant drought resistance of growth, midday leaf water potentials under moderate drought and species association to habitat dryness (Fig. 4), following hypothesized directions (Table1). However, for most traits the relations were not significant, and for few opposite to the hypotheses (Fig. 4).

Consistently, the results from the random forest models showed that the importance of different traits varied tremendously within each drought performance parameter, and at the same time different traits were the most important for different drought performance parameters (Fig. 4). This was underlined by the general lack of significantly positive rank correlations of trait importance between the drought performance parameters (Fig. 5). Only for drought resistance of survival and midday leaf water potential under moderate drought there was a significantly positive correlation of the ranks of trait importance, showing that the trait importance was overall consistent for these two parameters (Spearman's rho = 0.34, $p < 0.05$, Fig. 5).

For drought resistance of both survival and growth, root traits that maximize water uptake had the largest and significant importance, specifically high maximum rooting depth in dry treatment plots ($R_{dep\ dry}$) for survival and high root mass in dry treatment plots ($R_{mas\ dry}$) for growth (Fig. 4 A and B). For midday leaf water potentials under moderate drought, maintaining high leaf conductance under moderate drought ($g_{drought}$) was the most important (Fig. 4 C) with the effect direction opposite to the hypothesis (Table 1). High leaf water potential at turgor loss point (Ψ_{tlp}) which minimizes water loss under drought and low density of major leaf vein (VD_{major}) were the second and third important traits for midday leaf water potentials under moderate drought, respectively, with the effect direction of major leaf vein density again opposite to the hypothesis (Fig. 4C, Table 1). For species association to habitat dryness, yet other traits, namely a high stomatal index (ST_{ind}) and a small conduit diameter of minor leaf vein ($LV_{dia\ minor}$) were more important, suggesting a key role of stomatal regulation of transpiration and maintaining leaf hydraulic conductance under drought (Fig. 4D). Traits associated with water storage, membrane vulnerability or carbon gain were not significant in their importance for any of the drought performance parameters.


A DR survival
B DR growth
C MWP
D D ass
expected relations
tested relations
Water uptake
Water transport
Water storage
Water loss
Membrane vulnerability
Carbon gain
log R dap dry
log R dap irr
log R mas dry
log R mas irr
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log R TL irr
R MR dry
R MR irr
log R dia
log R SL
log R den
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log P RM
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log ST den
ST ind
log ST tcu
log SLA
log Ψ 88%SAL
A max
WUE inst
WUE mtr
log N mass
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1.0
0.5
0.0
-0.5
-1.0
0.0000
0.0004
0.0008
0.0000
0.0005
0.000
0.075
0.150
0.00
0.15
0.30

Fig. 4 The importance of traits for each drought performance parameters: (A) whole-plant drought resistance of survival ($DR_{survival}$), (B) whole-plant drought resistance of growth (DR_{growth}), (C) midday leaf water potentials under moderate drought (MWP) and (D) species association to habitat dryness (D_{ass}) across all species. Given are variable importance values assessed and tested using permutation-based random forest models (filled circles). Traits with significant importance (α = 0.05 after Bonferroni correction) are highlighted in black. Hypothesized directions of trait effects are shown as colored squares (green positive, red negative) and tested based on univariate regressions. Significance of correlation coefficients from univariate regression are given as *** P < 0.001, ** P < 0.01, * P < 0.05. See Table S3 for details of univariate regressions. Note that comparisons of variable importance values are useful to compare relative trait contributions *within* each data set, but not to evaluate the absolute trait contributions *across* data sets.

Differences between forbs and grasses in trait importance for drought performance parameters

Many traits (19 out of 38) differed significantly between forbs and grasses (Table 1). These includes traits that emerged as important for whole-plant drought resistance of survival and midday leaf water potentials under moderate drought, e.g. forbs had higher maximum rooting depth under dry conditions ($R_{dep\ dry}$) and higher leaf conductance under moderate drought ($g_{drought}$) than grasses. The trait differences were strong enough to separate forbs and grasses along PC 1 and PC 2 (Fig. 3). Forbs also maintained significantly higher midday leaf water potentials under moderate drought than grasses, while whole-plant drought resistance of both survival and growth, and species association to habitat dryness did not differ between forbs and grasses (Table 1).

Similar to the results across all species, within both forbs and grasses the trait importance also varied within each drought performance parameter and across them (Fig. 6). Only within grasses the ranks of trait importance of whole-plant drought resistance of survival were significantly positively related to midday leaf water potentials under moderate drought (Fig. 5). Between forbs and grasses, trait importance differed for each drought performance parameter, as indicated by the lack of significant rank correlations (Fig. 5).

Specifically, in forbs low minimum leaf conductance (g_{min}) and high leaf water potential at stomatal closure ($\Psi_{stclosure}$) both minimizing water loss under drought were the most important traits for whole-plant drought resistance of survival, followed by high root mass in dry treatment plots ($R_{mas\ dry}$) and thin roots (R_{dia}) that facilitate water uptake (Fig. 6A). In grasses high maximum rooting depth in dry treatment plots ($R_{dep\ dry}$) optimizing water uptake under

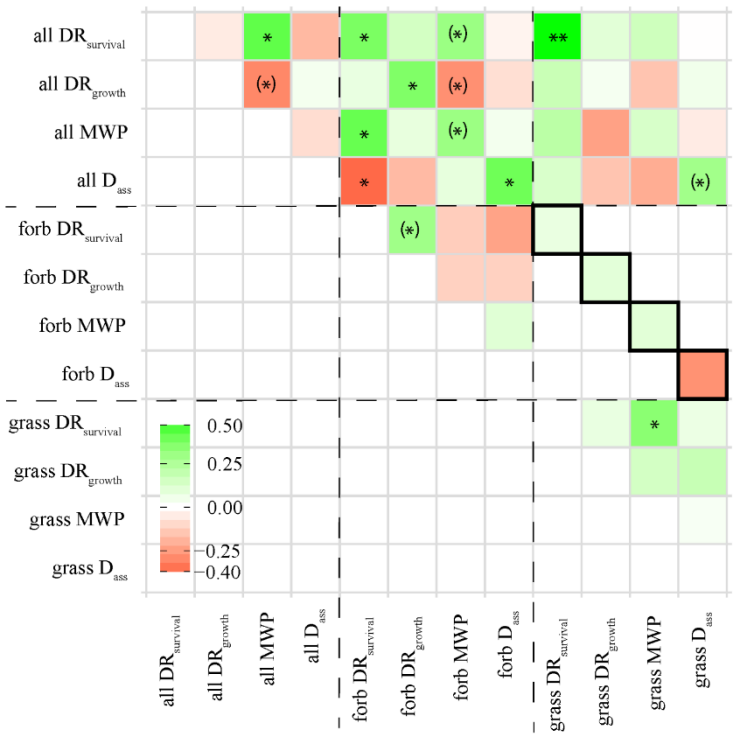

Fig. 5 Spearman rank correlations between trait importance for each drought performance parameters, i.e. whole-plant drought resistance of survival ($DR_{survival}$) and growth (DR_{growth}), midday leaf water potential under moderate drought (MWP) and species association to habitat dryness (D_{ass}) across all species, forbs and grasses. Significance of the relations is given as ** $P < 0.01$, * $P < 0.05$, (*) $P < 0.1$ with positive relations given in green and negative ones in red. A lack of significant relations indicates that the ranks of trait importance differ between drought performance parameters or between species groups, while significant relations indicate that the trait importance is related. Note that most relations are not significant. The comparisons of trait importance between forbs and grasses for different drought performance parameters are highlighted with black frames.

drought was the only significantly important trait for whole-plant drought resistance of survival (Fig. 6A). For whole-plant drought resistance of growth, high maximum photosynthesis (A_{max}) was the most important in forbs, while in grasses a low leaf C/N ratio (Fig. 6B). For high midday leaf water potentials under moderate drought, in grasses again high maximum rooting depth in dry treatment plots was the most important, while in forbs maintenance of high stomatal conductance under moderate drought ($g_{drought}$) and high leaf water potentials at turgor loss point (Ψ_{tlp}) had the higher importance than others (Fig. 6C). For association to habitat dryness, a high stomatal index was consistently the most important in both forbs and grasses (Fig. 6 D), consistent with the result across all species (Fig. 4 D), although the ranks of importance of other traits were unrelated between life forms (Fig. 5).

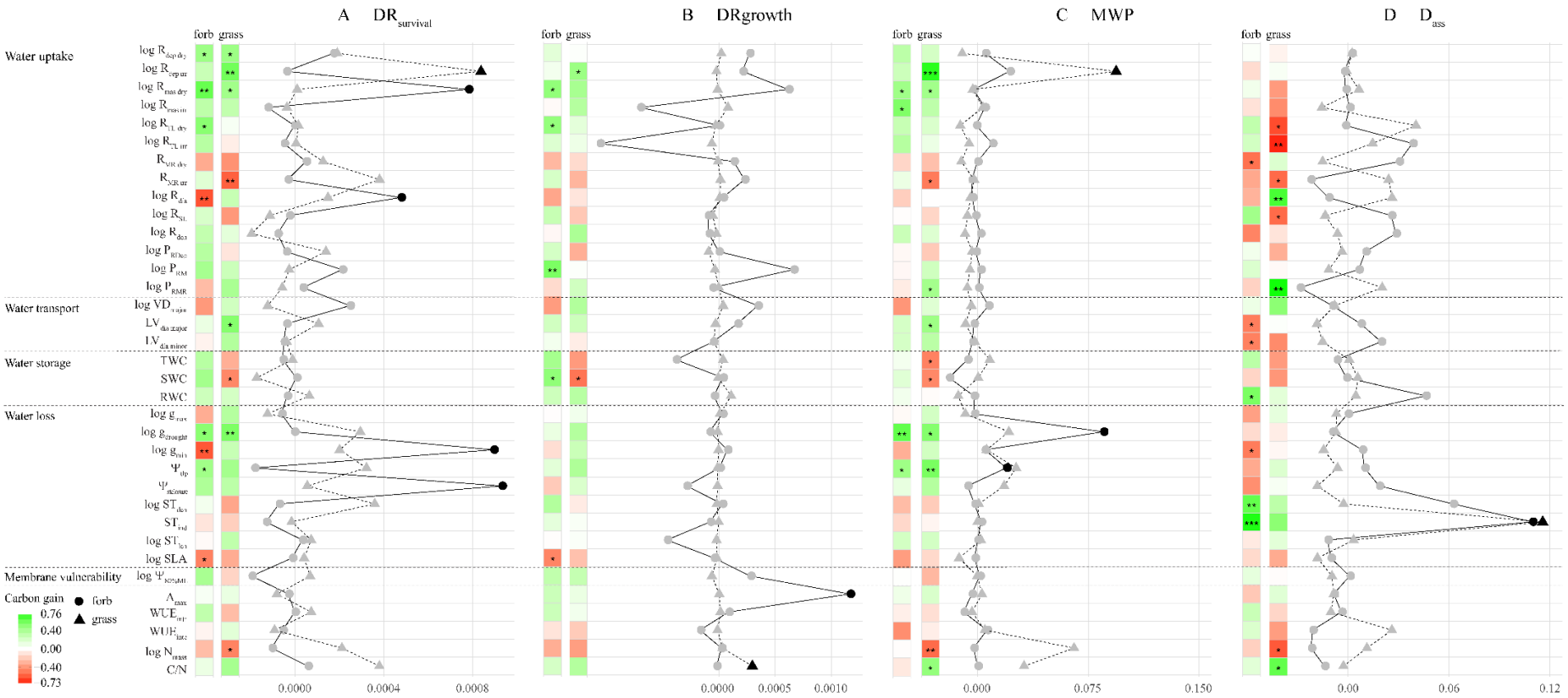

Fig. 6 Comparison between forbs and grasses of the trait importance for (A) whole-plant drought resistance of survival ($DR_{survival}$), (B) whole-plant drought resistance of growth (DR_{growth}), (C) midday leaf water potentials under moderate drought (MWP) and (D) species association to habitat dryness (D_{ass}). Given are variable importance values based on permutation-based random forest models with traits highlighted for their significant importance, and results of univariate regressions. For hypothesized directions of trait effects see Fig. 4. Note that direct comparisons of variable importance values between forbs and grasses are not useful.

Discussion

Coordination and trade-offs among traits

A large number of coordination and trade-offs existed among traits pertaining to water uptake, water transport, water storage, water loss, membrane vulnerability and carbon gain processes (Fig. 1-3). This supports that multiple traits in coordination, not alone, determined species drought resistance in grasslands. Relations of subset of these traits have been shown in previous studies (Jackson *et al.*, 1997; Craine *et al.*, 2001; Tucker *et al.*, 2011; Zwicke *et al.*, 2015; Bartlett *et al.*, 2016; Lawson & McElwain, 2016), but never involving such a large number of traits which allows to comprehensively evaluate the coordination and trade-offs among traits relevant for plant water relations and hypothesized to be important for drought resistance. Some of the correlations can be explained by direct physiological linkages or biophysical constrains. This is true for the positive correlations between leaf water potentials at turgor loss point and stomatal closure (McAdam & Brodribb, 2015) or between leaf nitrogen content and integrated water use efficiency (Sparks & Ehleringer, 1997), for the negative correlation between stomatal density and stomatal size (de Boer *et al.*, 2016), or for the relations between specific root length, root diameter, root tissue density, root total length and root phenotypic plasticity in response to drought (Eissenstat, 1992; Reich *et al.*, 1999; Comas *et al.*, 2013). Other trait relations we found reflected the co-selection of different processes under drought resulting in their coordination (Westoby & Wright, 2006). For example, increased water uptake (i.e. through high maximum rooting depth) is most beneficial under drought conditions if coordinated with an efficient water transport system to transport and distribute water (i.e. through high density of minor leaf veins) and concurrent with sensitive stomata that close early under drought or high vapor pressure deficit conditions (i.e. through high leaf water potentials at stomatal closure, Fig. 1).

At the same time, trait correlations were overall weak, many traits within the same process and across processes were unrelated to each other (Fig. 1) and no dominating axes of variation of the traits emerged (Fig. 1 and Fig. 3), suggesting that considerable freedom in combination of traits related to drought resistance existed in grassland species. Weak or non-significant correlations between traits relevant for drought resistance were also found in other grassland studies (Craine *et al.*, 2001; Tucker *et al.*, 2011; Zwicke *et al.*, 2015; Belluau & Shipley, 2018).

Together, these results support that grassland plants exhibit a multitude of combinations of traits, which confer the overall high drought resistance of individual grassland species.

At the global scale, trait coordination at leaf level both in herbaceous grassland species and in woody species can be simplified by the leaf economics spectrum (LES) into 'fast' or 'slow' growth traits (Wright *et al.*, 2004). Later studies have extended the LES to leaf vein traits (Sack *et al.*, 2013), to stem (Chave *et al.*, 2009) and root traits (Craine *et al.*, 2005), and to whole-plant level (Reich, 2014). The LES was shown to be associated with species shade tolerance (Kitajima & Poorter, 2010; Poorter *et al.*, 2010; Wright *et al.*, 2010) and has been hypothesized to also be associated with drought resistance (Reich, 2014): traits associated with 'slow' growth such as high root tissue density, low specific root length, low specific leaf area, low leaf nitrogen content, and low maximum photosynthesis, should be coordinated and related to high drought resistance. However, although some pair-wise correlations among the proposed traits were found in our study and in other grassland communities (Craine *et al.*, 2005; Tjoelker *et al.*, 2005; Tucker *et al.*, 2011; Maire *et al.*, 2012), an overall coordination at whole-plant level along main axes did not emerge for these traits in our study. This is in line with results from studies at similar scales, i.e. at regional (Bergmann *et al.*, 2017) or local scale (Craine *et al.*, 2001; Tjoelker *et al.*, 2005; Tucker *et al.*, 2011; Maire *et al.*, 2012; Perez-Ramos *et al.*, 2013; Zhou *et al.*, 2018). The lack of pronounced trait coordination, which we had expected based on LES, may be, at least partly, due to the much smaller trait variation at local and regional scales than the global scale of the LES, e.g. the global variance in N_{mass} of grassland species was 1.16% (Wright *et al.*, 2004) but only 0.50% for our species. However, Craine *et al.* (2005) found that for grassland species even though at global scale the LES existed for leaf traits or root traits, a coordination across plant organs was absent, and that high, rather than low, leaf nitrogen content enabled species to inhabit dry habitats, contrary to the slow growth 'strategy' (but see Table 1). Consistently, in our common garden experiment (Manuscript 1) and in previous studies (Fernandez & Reynolds, 2000), no support was found for the trade-off between optimal growth and drought resistance in grassland species. These results suggest that a coordination between 'slow' growth traits may not exist and a slow growth 'strategy' - or the traits associated to slow growth - therefore may be overall not important for the drought resistance of grassland species. The inability to simply trait relations suggest that we may need to combine several traits to improve our prediction of differential species drought resistance (Choat *et al.*, 2018).

Differential importance of traits for drought performance parameters

To our knowledge, our study is the first that evaluated the importance of such a comprehensive set of traits pertaining to all the processes (see above) for several drought performance parameters comparatively across multiple grassland species. Many traits in our study pertaining to different processes were significantly correlated with the drought performance parameters (Fig. 4), suggesting that multiple processes contribute to promoting species drought resistance and supporting our argument that the prediction of drought resistance may involve several traits (see above). However, although all traits have been hypothesized or shown to be important for drought resistance (Table 1), they differed strongly in their importance for species drought performances with few traits having high importance (Fig. 4). For whole-plant drought resistance of both survival and growth, traits maximizing water uptake through deep roots or high root biomass had the highest importance. This is consistent with previous studies focusing on fewer traits where contrasted with other traits, significant positive correlations between maximum rooting depth/root mass and whole-plant drought resistance of survival or growth were found (Perez-Ramos *et al.*, 2013; Zwicke *et al.*, 2015; Manuscript 3), supporting the crucial role of roots in promoting whole-plant drought resistance in grassland species. Many traits have been shown to be important in determining species distribution, such as gas exchange traits (Belluau & Shipley, 2017), leaf water potentials at stomatal closure (Tucker *et al.*, 2011; Craine *et al.*, 2013), or leaf or root morphological traits (Tucker *et al.*, 2011; Shipley *et al.*, 2017). Here, we found that compared with these traits, high stomatal index had the highest importance for species habitat dryness while its importance for other drought performance parameters is minor (Fig. 4). Opposite to our expectation, high, rather than low, stomatal conductance under moderate drought ($g_{drought}$) contributed mostly to high midday leaf water potentials under drought (Fig. 4). High $g_{drought}$ also promoted high whole-plant drought resistance of survival (Fig. 4). These results are counterintuitive because (1) high $g_{drought}$ allows water loss under drought, and, therefore, should lead to low leaf water potentials under drought and low whole-plant drought resistance of survival; (2) high leaf water potentials at turgor loss point and at stomatal closure which were important for both of these parameters should lead to low $g_{drought}$. A possible explanation resolving these apparent contradictions could be that species with $g_{drought}$ also had high rooting depth (Fig. 2O). Previous study showed that high rooting depth could enable plants to maintain high stomatal conductance without decreases in leaf water potentials under drought (Tuzet *et al.*, 2003). Further, $g_{drought}$ was measured in the morning under moderate drought. High leaf water potentials at turgor loss point and at stomatal

closure can temporarily depress stomatal conductance at midday to minimize water loss when vapor pressure deficit and transpiration are high (Bartlett *et al.*, 2012b). In the morning, the high leaf water potentials would in turn lead to high $g_{drought}$ under moderate drought (Tuzet *et al.*, 2003). But under severe or lasting drought, permanent wilting or stomatal closure may occur in the morning and result in low $g_{drought}$. Further studies are needed to validate these relations. A practical implication of the differential trait importance is that for predicting drought resistance, this would allow us to simplify the traits measurement routine by selecting these important traits. For example, in manuscript 3, we found that by including important root traits, only three traits can provide high predictive power of whole-plant drought resistance of survival for both forbs and grasses ($r^2 \geq 56\%$), although different trait combinations were used for each group.

We expected that the same trait combinations would allow plants to maintain high midday leaf water potentials under drought, confer high drought resistance of survival and growth and enable plants to inhabit dry habitats. However, as shown above, the traits with the highest importance for each drought performance parameter were different. Moreover, only for whole-plant drought resistance of survival and midday leaf water potentials under drought, a significantly positive correlation between the trait importance ranks was found (Fig. 5), supporting that maintenance of high leaf water potentials is important for survival under drought stress in grassland species (Choat *et al.*, 2018; Manuscript 2). For whole-plant drought resistance of growth and association to habitat dryness, different traits emerged as important. Indeed, the relation between growth and survival under drought is not straightforward. On one hand, whole-plant drought resistance of growth may exhibit a trade-off with whole-plant drought resistance of survival because reduced growth or leaf mortality can promote survival by decreasing transpiration area (Volaire *et al.*, 1998; Volaire & Norton, 2006). On the other hand, whole-plant drought resistance of growth and survival can be positively related when leaf mortality precedes plant death (Rivero *et al.*, 2007; Zwicke *et al.*, 2015). The relation between whole-plant drought resistance experimentally assessed in our study and species association to habitat dryness was weak (Manuscript 1), suggesting that species whole-plant drought resistance may have only a limited role in determining distribution patterns. Other environmental factors such as grazing, nutrient and light, and biotic interactions among plants additionally influence plant performance in grasslands and interact in complex ways, contributing to shaping abundance and distribution patterns in grasslands (Proulx & Mazumder, 1998; Maron & Crone, 2006; Borer *et al.*, 2014; Breitschwerdt *et al.*, 2018). In

summary, the generalization of traits underlying drought resistance is impeded by the weak relations between different drought performance parameters. The selection of traits for the predictions of drought resistance, therefore, should also consider the drought performance parameters that are relevant for the research questions.

Specific leaf area (SLA) has been extensively used in trait-based studies on drought responses based on the assumption that species with low SLA have low transpiration rate and thus high drought resistance, and because it is one of the most easily measured and widely available traits (Westoby *et al.*, 2002; Diaz *et al.*, 2004; Kattge *et al.*, 2011; Díaz *et al.*, 2015). The association of SLA with drought resistance has been tested in grassland species, however with inconsistent results: a low SLA although was found to be highly related to high leaf water potentials under drought (Perez-Ramos *et al.*, 2013), but unrelated to whole-plant drought resistance of survival and growth (Weißhuhn *et al.*, 2011; Perez-Ramos *et al.*, 2013; Zwicke *et al.*, 2015). SLA was unrelated or only weakly related to habitat dryness (Cingolani *et al.*, 2007; Belluau & Shipley, 2018) or with opposite direction to habitat dryness for forbs and grasses (Shipley *et al.*, 2017). In our study, SLA had relative low importance for all drought performance parameters, was only weakly negatively related with whole-plant drought resistance of survival and growth, and unrelated to species association to dry habitats or midday leaf water potentials under drought (Fig. 4). The overall weak or insignificant relations and inconsistent results suggest that SLA may not be a suitable and widely applicable proxy for species drought resistance.

Functional differences between forbs and grasses

We found that many traits significantly differed between forbs and grasses, including the traits that were important for the drought performance parameters (Table 2, Fig. 3). The differences between forbs and grasses in their traits have been also validated in individual studies with subset of traits (Grime *et al.*, 1997; Craine *et al.*, 2001; Nippert & Knapp, 2007; Tucker *et al.*, 2011; Chen *et al.*, 2017). Further, the ranks of trait importance between forbs and grasses for each drought performance parameters were unrelated (Fig. 5). Overall, these data support substantial functional differences between forbs and grasses. However, no significant differences in drought performances parameters were found between these two groups, except midday leaf water potentials under drought, suggesting that both groups may suffer from the projected drought. This is contrasted with previous studies showing that forbs had lower (Tilman & El Haddi, 1992; Skinner & Comas, 2010) or higher (Yang *et al.*, 2011) drought resistance than grasses. Analyzing 20 forbs and 21 grasses in manuscript 1, we found that

species whole-plant drought resistance differed significantly within forbs as well as within grasses. Both life forms contained drought resistance and drought sensitive species. The different species compositions used in these studies (Tilman & El Haddi, 1992; Skinner & Comas, 2010; Yang *et al.*, 2011) thus may cause the inconsistence. In summary, large functional differences between forbs and grasses were found. Thus, the relations between traits and drought resistance, and the trait importance should be assessed individually for different life forms and phylogenetic groups.

Conclusions

The relations between traits that are relevant for plant drought resistance are complex. Various trait combinations involving several processes existed for forbs and grasses to promote different drought performance parameters, and no single axis emerged to define the spectrum of drought resistance. These complexities make predictions of species responses to drought challenging. However, many traits showed low and insignificant importance which reduces the complexity and simplifies the prediction models. Our study involving a large number of traits and drought performance parameters across multiple species thus can provide practical guides for trait selection for the prediction of changes in grassland composition and ecosystem services under climate change.

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Supporting information

Table S1 List of the 41 temperate grassland species in our study with family, life form and association to habitat dryness (D_{ass}).

Species	Family	Life form	D_{ass}
Achillea millefolium L.	Asteraceae	Forb	-4
[a]*Agrostis capillaris* L.	Poaceae	Grass	x
Agrostis stolonifera L.	Poaceae	Grass	-7
Alopecurus pratensis L.	Poaceae	Grass	-6
Anthoxanthum odoratum L.	Poaceae	Grass	x
Arrhenatherum elatius (L.) P.Beauv. ex J.Presl & C.Presl.	Poaceae	Grass	x
Brachypodium pinnatum (L.) P.Beauv.	Poaceae	Grass	-4
Briza media L.	Poaceae	Grass	x
Bromus hordeaceus L.	Poaceae	Grass	x
Centaurea jacea L.	Asteraceae	Forb	x
Cerastium holosteoides Fr.	Caryophyllaceae	Forb	-5
Cirsium oleraceum (L.) Scop.	Asteraceae	Forb	-7
Crepis biennis Lapeyr.	Asteraceae	Forb	-6
Dactylis glomerata L.	Poaceae	Grass	-5
Daucus carota L.	Apiaceae	Forb	-4
[a]*Elymus repens* (L.) Gould	Poaceae	Grass	x
[ab]*Festuca guestfalica* Boenn. ex Rchb.	Poaceae	Grass	-4
[ab]*Festuca ovina* L.	Poaceae	Grass	x
Festuca pratensis Huds.	Poaceae	Grass	-6
[b]*Festuca rubra* L.	Poaceae	Grass	-6
[a]*Galium mollugo* L.	Rubiaceae	Forb	-4
Helictotrichon pubescens (Huds.) Schult. & Schult.f.	Poaceae	Grass	-3
Holcus lanatus L.	Poaceae	Grass	-6
Lathyrus pratensis L.	Fabaceae	Forb	-6
Leontodon autumnalis L.	Asteraceae	Forb	-5
[a]*Leucanthemum vulgare* (Vaill.) Lam.	Asteraceae	Forb	-4
Lolium perenne L.	Poaceae	Grass	-5
Lotus corniculatus L.	Fabaceae	Forb	-4
Phleum pretense L.	Poaceae	Grass	-5
Plantago lanceolata L.	Plantaginaceae	Forb	x
Poa pratensis L.	Poaceae	Grass	-5
[b]*Poa trivialis* L.	Poaceae	Grass	-7
Prunella vulgaris L.	Lamiaceae	Forb	-5
Ranunculus acris L.	Ranunculaceae	Forb	-6
Ranunculus bulbosus L.	Ranunculaceae	Forb	-3
Ranunculus repens L.	Ranunculaceae	Forb	-7
Rumex acetosa L.	Polygonaceae	Forb	x
Rumex crispus L.	Polygonaceae	Forb	-7
Taraxacum officinale (L.) Weber ex F.H.Wigg.	Asteraceae	Forb	-5
Trisetum flavescents (L.) P. Beauv.	Poaceae	Grass	x
Vicia cracca L.	Fabaceae	Forb	-6

Species association to habitat dryness (D_{ass}) was based on Ellenberg's soil moisture indicator values (Ellenberg *et al.*, 1991) multiplied by -1 so that higher values indicate higher association to drier habitats, consistent with the direction of values of whole-plant drought resistance of survival and growth (see main text). x indicates that species without specific moisture requirements and these species (11 in total) were excluded from analyses concerning habitat

association. Note: [a] and [b] indicate that root depth distribution and total and minor leaf vein density were not assessed on these species, respectively (see Methods).

Table S2 Information about the plants that were used for the measurements, i.e. growing conditions (common garden or greenhouse), number of individuals per species measured for each trait and sets of plants on which traits were measured in the greenhouse (A-L).

Traits	Conditions	Number of individuals	Set of plants
Performance under drought			
$DR_{survival}$	common garden	36	
DR_{growth}	common garden	6	
MWP	common garden	8	
D_{ass}	published data		
Water uptake			
$R_{dep\ dry}$	common garden	8	
$R_{dep\ irr}$	common garden	8	
$R_{dep\ dis}$	published data	1	
$R_{mas\ dry}$	common garden	8	
$R_{mas\ irr}$	common garden	8	
$R_{TL\ dry}$	common garden	8	
$R_{TL\ irr}$	common garden	8	
$R_{MR\ dry}$	common garden	8	
$R_{MR\ irr}$	common garden	8	
R_{dia}	green house	3	A
R_{SL}	green house	3	A
R_{den}	green house	3	A
P_{RDep}	common garden	8	
P_{RM}	common garden	8	
P_{RMR}	common garden	8	
Water transport			
VD_{total}	green house	6	B
VD_{major}	green house	6	B
VD_{minor}	green house	6	B
$LV_{dia\ major}$	green house	6	C
$LV_{dia\ minor}$	green house	6	C
Water storage			
TWC	green house	3	D
SWC	green house	3	D
RWC	green house	3	D
Water loss			
g_{max}	green house	6	E
$g_{drought}$	common garden	8	
g_{min}	green house		F
ψ_{tlp}	green house	6	G

$\psi_{stclosure}$	green house	6	H
ST_{den}	green house	6	I
ST_{ind}	green house	6	I
ST_{len}	green house	6	I
SLA	green house	6	J
membrane vulnerability			
$\psi_{80\%ML}$	green house	10-15	K
carbon gain			
A_{max}	green house	6	E
WUE_{intr}	green house	6	E
WUE_{inte}	green house	6	L
N_{mass}	green house	6	L
C/N	green house	6	L

Trait measurements that used the same set of plants in the greenhouse were indicated by the same letter/letters. For details of trait measurements see Methods and Materials. For full trait names, units, the hypothesized relations to the drought resistance and summary statistics see Table 1 and Table 2.

Table S3 Relations of traits with whole-plant drought resistance of survival ($DR_{survival}$), whole-plant drought resistance of growth (DR_{growth}), midday leaf water potential under moderate drought (MWP) and species association to habitat dryness (D_{ass}).

	all species				forbs				grasses			
traits	$DR_{survival}$	DR_{growth}	MWP	D_{ass}	$DR_{survival}$	DR_{growth}	MWP	D_{ass}	$DR_{survival}$	DR_{growth}	MWP	D_{ass}
Water uptake												
$\log R_{Dep\ dry}$	0.46**	0.11	0.37*	0.02	0.45*	0.15	0.33	0.02	0.46*	0.02	0.22	-0.05
$\log R_{Dep\ irr}$	0.39*	0.15	0.32*	-0.09	0.27	0.06	0.26	-0.14	0.60**	0.46*	0.74***†	0.04
$\log R_{Mas\ dry}$	0.52**†	0.46**	0.35*	-0.12	0.63**	0.50*	0.45*	0.07	0.44*	0.42	0.44*	-0.39
$\log R_{Mas\ irr}$	0.20	0.06	0.03	-0.23	0.33	-0.02	0.56*	-0.11	0.31	0.36	0.32	-0.45
$\log R_{TL\ dry}$	0.21	0.35*	-0.02	-0.06	0.53*	0.49*	0.28	0.31	0.04	0.14	0.19	-0.65*
$\log R_{TL\ irr}$	0.02	0.10	-0.20	-0.12	0.35	0.18	0.36	0.23	-0.07	0.09	0.10	-0.73**
$R_{MR\ dry}$	-0.35*	-0.26	-0.23	-0.35	-0.33	-0.28	-0.16	-0.54*	-0.43	-0.14	-0.25	0.18
$R_{MR\ irr}$	-0.18	0.10	-0.21	-0.39*	0.16	0.21	0.10	-0.34	-0.59**	-0.29	-0.51*	-0.56*
$\log R_{Dia}$	0.00	-0.19	0.20	0.16	-0.63**	-0.35	-0.19	-0.30	0.28	-0.10	0.00	0.70**
$\log R_{SL}$	-0.16	0.11	-0.36*	0.02	0.20	0.28	-0.01	0.44	-0.40	-0.22	-0.17	-0.56*
$\log R_{Den}$	0.25	0.08	0.39*	-0.28	0.30	-0.03	0.27	-0.47	0.14	0.40	0.17	-0.11
$\log PRDep$	0.20	0.11	0.18	<0.01	0.36	0.22	-0.03	0.07	-0.10	-0.32	-0.21	-0.32
$\log PRM$	0.35*	0.37*	0.32*	0.12	0.42	0.57**	-0.03	0.19	0.26	0.01	0.24	-0.02
$\log PRMR$	-0.11	-0.11	-0.05	-0.07	-0.26	-0.14	-0.08	-0.18	0.41	0.24	0.43*	0.75**
Water transport												
$\log VD_{major}$	-0.16	-0.13	-0.48**	0.12	-0.40	-0.39	-0.41	0.09	0.24	0.38	0.17	0.52
$LV_{Dia\ major}$	0.20	0.20	-0.02	-0.30	0.11	0.23	0.22	-0.50*	0.54*	0.25	0.46*	0.02
$LV_{Dia\ minor}$	0.10	0.00	-0.10	-0.40*	-0.05	0.07	0.22	-0.49*	0.37	-0.07	0.34	-0.45
Water storage												
TWC	0.05	0.23	-0.13	0.04	0.35	0.43	0.06	0.34	-0.31	-0.40	-0.48*	-0.39
SWC	-0.03	0.15	0.00	-0.26	0.43	0.50*	0.16	-0.17	-0.47*	-0.53*	-0.46*	-0.41
RWC	0.20	0.27	-0.05	0.43*	0.26	0.26	0.07	0.58*	0.22	0.35	-0.01	0.15
Water loss												
$\log g_{max}$	0.06	0.02	0.07	-0.16	-0.32	-0.01	-0.02	-0.37	0.36	0.07	0.23	0.13
$\log g_{drought}$	0.51**†	0.15	0.69**†	-0.07	0.53*	0.11	0.64**	-0.14	0.57**	0.37	0.54*	-0.07
$\log g_{min}$	-0.03	0.00	0.31	-0.21	-0.62**	-0.13	-0.32	-0.50*	0.28	0.27	0.24	-0.03
ψ_{tlp}	0.45**	0.20	0.60**†	-0.07	0.45*	0.15	0.45*	-0.34	0.43	0.39	0.58**	0.18
$\psi_{stclosure}$	0.37*	-0.05	0.47**	-0.15	0.39	-0.21	0.12	-0.43	0.33	0.19	0.36	0.05
$\log ST_{Den}$	-0.08	0.26	-0.04	0.52**	0.07	0.37	-0.27	0.64**	-0.35	-0.08	-0.20	0.33
ST_{Ind}	-0.12	0.11	0.02	0.64***†	-0.10	0.14	-0.15	0.75***	-0.19	0.03	-0.04	0.52
$\log ST_{Len}$	0.16	0.00	0.23	0.02	-0.02	-0.01	0.23	-0.09	0.32	0.03	0.23	0.15
$\log SLA$	-0.37*	-0.36*	-0.14	-0.21	-0.48*	-0.47*	-0.38	-0.15	-0.33	-0.24	-0.15	-0.33
Membrane stability												

$\log \psi_{80\%ML}$	0.19	0.31	-0.01	0.10	0.41	0.31	0.01	0.13	-0.17	0.29	-0.28	-0.01
Carbon gain												
A_{max}	0.09	0.23	0.20	-0.09	0.04	0.26	0.09	-0.22	0.11	0.11	0.20	0.16
WUE_{Itrt}	0.00	0.18	-0.01	0.10	0.31	0.26	-0.06	0.27	-0.26	0.07	-0.13	-0.16
WUE_{Inte}	0.07	-0.12	-0.18	-0.06	-0.04	-0.11	-0.44	0.19	0.15	-0.21	-0.07	-0.38
$\log N_{mass}$	-0.34*	-0.31	-0.16	-0.31	-0.26	-0.32	-0.01	-0.19	-0.48*	-0.38	-0.56**	-0.61*
C/N	0.25	0.23	0.11	0.32	0.10	0.22	-0.09	0.15	0.42	0.37	0.49*	0.64*

Given are correlation coefficients from univariate regressions and corresponding significance. * P values < 0.05, ** P values < 0.01, *** P values < 0.001. Note † indicates that relations are still significant after Bonferroni adjustment at $\alpha = 0.05$.

List of manuscripts and specification of own contributions

Manuscript 1

Authors	Eun-Young Jung (EJ), Julian Gaviria (JG), Shanwen Sun (SS), Bettina M.J. Engelbrecht (BMJE)
Title	Comparative drought resistance of temperate grassland species: evaluation of performance trade-offs and the relation to species distribution across moisture gradients
Status	prepared for submission to Oikos (reformatted for the thesis)
Contributions	The study was designed by BMJE with additions by EJ, JG, and SS. The experiment was set up and data were collected by JG and EJ, with support from SS, colleagues, students and assistants (see acknowledgements). The data were analyzed, and figures and tables created by EJ with suggestions from SS and BMJE. Results were discussed by EJ, SS and BMJE. EJ wrote the manuscript with revisions and rewriting of the manuscript mainly by BMJE and SS. EJ is the corresponding author.

Manuscript 2

Authors	Sun Shanwen, Eun-Young Jung, Julian Gaviria, Bettina M. J. Engelbrecht
Title	High turgor loss point promotes drought survival of temperate grassland species
Status	Submitted to New Phytologist (with positive comments). revised for submission to Plant, Cell & Environment (reformatted for the thesis)
Contributions	The study was designed by SS and BMJE with modifications by EJ and JG. Physiological measurements were performed by SS, and the field experiment set up and maintained by JG, with support from colleagues, students and assistants (see acknowledgements). The data were analyzed, and figures and tables created by SS with suggestions from BMJE and EJ. SS, EJ, JG and BMJE interpreted and discussed the results. SS wrote the first draft of the manuscript, with extensive revision and rewriting of the manuscript by BMJE and further input by SS, EJ, JG. SS is the corresponding author.

Manuscript 3

Authors	Sun Shanwen, Eun-Young Jung, Bettina M. J. Engelbrecht
Title	Root traits and turgor loss point explain differential drought resistance in temperate grassland species
Status	preparation for publication in Functional Ecology
Contributions	The study was designed by SS and BMJE with input by EJ. SS performed trait measurements with support from students and assistants (see acknowledgements). The field experiment was set-up and maintained by colleagues with support (see acknowledgements and manuscripts 1 and 2). The data were analyzed, and figures and tables were created by SS with suggestions from BMJE and EJ. SS, EJ and BMJE interpreted and discussed the results. SS wrote the first draft of the manuscript, with extensive revision and rewriting of the manuscript by BMJE and further input by SS and EJ. SS finalized the submitted version and will act as corresponding author.

Manuscript 4

Authors	Sun Shanwen, Eun-Young Jung, Bettina M. J. Engelbrecht
Title	Trait coordination and importance for drought performance in temperate grassland species
Status	prepared for publication in PLOS ONE
Contributions	The study was designed by SS and BMJE with input by EJ. SS performed trait measurements with support from students and assistants (see acknowledgements). The field experiment was set-up and maintained by colleagues with support (see acknowledgements and manuscripts 1 and 2). The data were analyzed, and figures and tables were created by SS with suggestions from BMJE and EJ. SS, EJ and BMJE interpreted and discussed the results. SS wrote the first draft of the manuscript, with extensive revision and rewriting of the manuscript by BMJE and further input by SS and EJ. SS finalized the submitted version and will act as corresponding author.

Additional publication not included in this thesis

Megan K. Bartlett, Christine Scoffoni, Rico Ardy, Ya Zhang, Shanwen Sun, Kunfang Cao, Lawren Sack (2012). Rapid determination of comparative drought tolerance traits: using an osmometer to predict turgor loss point. Methods in Ecology and Evolution 3: 880–888.

Shanwen Sun, Yongjiang Zhang, and Kunfang Cao. (2014). The correlation between leaf structure, drought tolerance and photosynthetic capacity in saplings of Euphorbiaceae from different micro-habitats in a seasonal tropical rainforest. Chinese Journal of Plant Ecology 38(4): 311-324

Megan K. Bartlett, Ya Zhang, Nissa Kreidler, Shanwen Sun, Rico Ardy, Kunfang Cao, and Lawren Sack. (2014). Global meta-analysis of plasticity in turgor loss point, a key drought tolerance trait. Ecology letters 17(12): 1580-1590.

Megan K. Bartlett, Ya Zhang, Jie Yang, Nissa Kreidler, Shanwen Sun, Luoxiang Lin, Yuehua Hu, Kunfang Cao, and Lawren Sack. (2016). Drought tolerance as a driver of tropical forest assembly: resolving spatial signatures for multiple processes. Ecology 97(2): 503-514.

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